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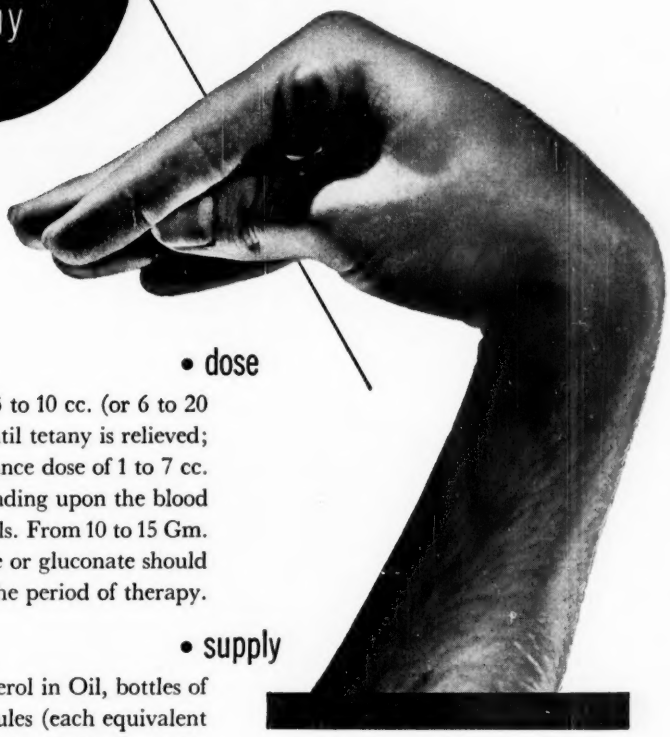

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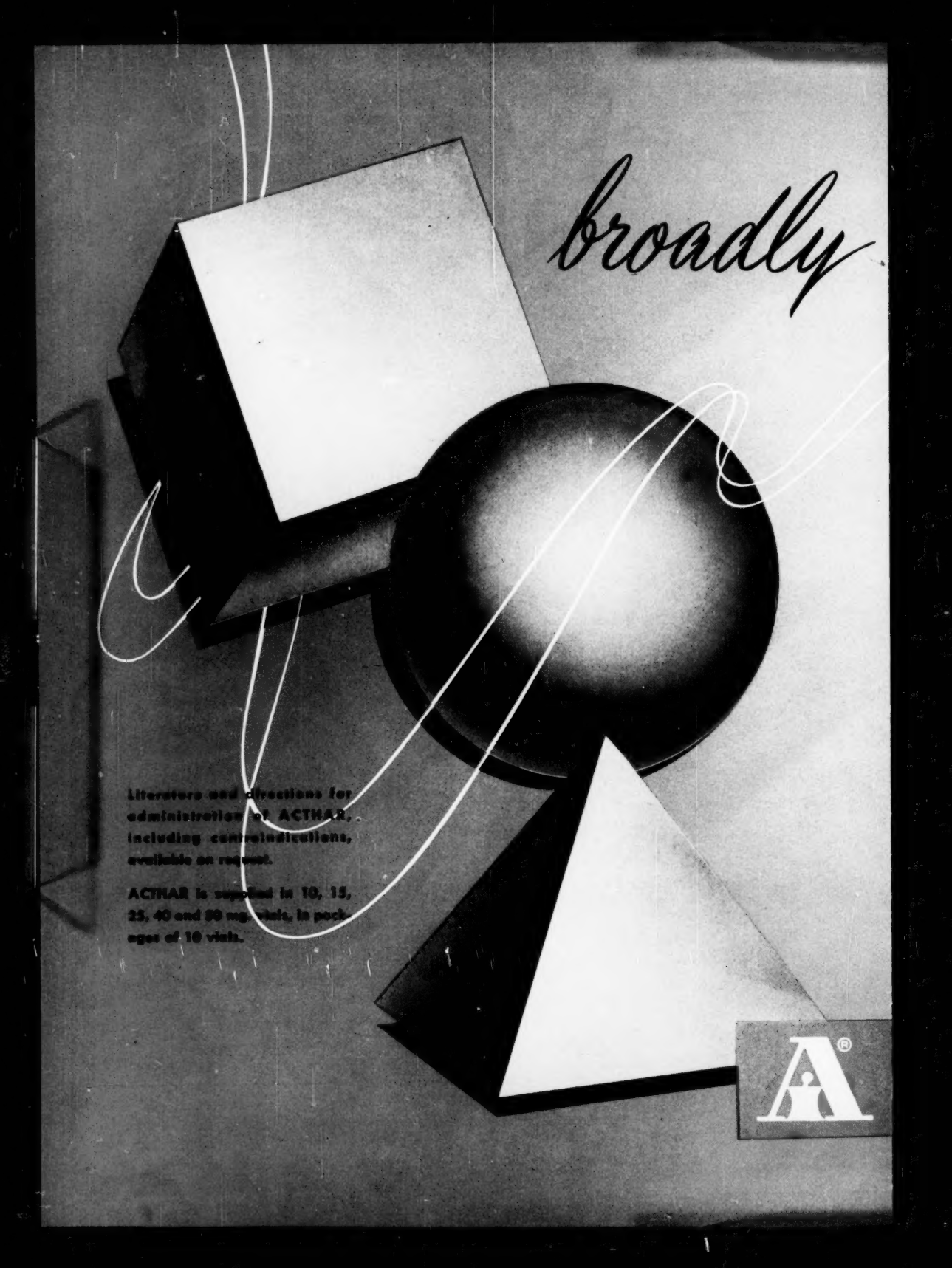
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EFFECT OF DIABETES AND INSULIN ON THE MAXIMUM CAPACITY OF THE RENAL TUBULES TO REABSORB GLUCOSE [1]

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(Submitted for publication September 5, 1950; accepted, October 30, 1950)

Glycosuria occurs whenever the amount of glucose filtered by the glomeruli exceeds the reabsorptive capacity of the tubules. The glycosuria of diabetes, therefore, is dependent not only on the degree of hyperglycemia but also on the concurrent glucose reabsorptive capacity.

A number of observers have noted that diabetic patients sometimes may have little or no glycosuria in spite of hyperglycemia (1, 2). Mirsky and Nelson (2) have attributed this phenomenon to decreased glomerular filtration rate, especially among elderly patients, or to an increase in the glucose reabsorptive capacity of the tubules.

Glucose reabsorption depends on an active transport system which is undoubtedly enzymatic in nature (3) and, therefore, is limited by a maximum rate, designated Tm_G. This transport system appears to be subject to a variety of functional controls including hormones. Indeed, slight reduction in Tm_G after the administration of insulin has been observed in the dog (4), while thyroid extract has been noted to increase Tm_G (5).

The present study represents an evaluation in man of the effects of diabetes and of insulin on the maximum ability of the tubules to reabsorb glucose.

SUBJECTS AND METHODS

Glomerular filtration rate (GFR) and Tm_G were studied in 12 diabetic patients. Nine non-diabetic subjects served as controls. Pertinent clinical data are summarized in Table I. The duration of diabetes was not known with certainty in each instance, but was mild to moderate

[1] This work was supported by a grant from the U. S. Public Health Service.

TABLE I
Clinical data

Patient	Sex	Age	Surface area	Known duration diabetes	Insulin requirements	Fasting plasma glucose	Blood pressure	Urine	Fundi
			(sq. m.)	*(years)*	*(units per day)*	*(mgm. per cent)*	*(mm. Hg)*	*(protein)*	
1	M	49	1.62	1	10 PZI	120	110/74	0	normal
2	M	61	1.91	2	none	123	112/68	0	normal
3	M	37	1.75	7	35 PZI	204	120/90	5 mm. per cent	normal
4	M	38	1.83	3	40 PZI, 15 Reg.	287	128/70	0	slight sclerosis vessel
5	M	53	1.71	2	none*	368	160/80	0	normal
6	M	43	1.91	23	80 Reg.	455	120/80	0	normal
7	F	45	1.70	3	5 Reg.	115	130/80	0	normal
8	F	73	1.74	1	none	145	160/90	0	sclerosis vessels
9	F	65	1.64	9	15 PZI	159	140/90	0	sclerosis vessels
10	F	45	1.64	7	10 PZI	173	145/80	1 +	few patches exudate
11	F	43	1.62	3	5 Reg.	200	120/88	5 mm.%	slight A-V nicking
12	F	40	1.68	16	45 PZI, 20 Reg.	480	120/80	0	normal
13	M	43	1.65	Non-diabetic				normal	
14	M	27	1.87	Non-diabetic				normal	
15	M	53	1.72	Non-diabetic				normal	
16	M	48	1.95	Non-diabetic				normal	
17	M	23	1.66	Non-diabetic				normal	
18	M	47	1.74	Non-diabetic				normal	
19	F	44	1.78	Non-diabetic				normal	
20	F	28	1.52	Non-diabetic				normal	
21	F	25	1.67	Non-diabetic				normal	

* Resistant to subcutaneous insulin, treated by diet alone.

in severity as evidenced by the daily insulin requirements and the fasting plasma glucose levels. None of the diabetic patients had established hypertensive disease. Minimal proteinuria was observed in two patients, while one patient had slight proteinuria and a few patches of exudate in the fundi. Mild to moderate sclerosis of the retinal vessels was observed in four patients.

All experiments were performed in the post-absorptive state. Insulin therapy was witheld for 24 hours prior to a study. In each study Tm_G was measured for three or more consecutive periods. In 15 of the 21 studies, inulin clearance was measured for three 15 to 20 minute periods prior to the start of the glucose infusions. Priming doses of 50 per cent glucose in water were given intravenously in amounts calculated to raise the plasma glucose to between 500 and 800 mgm. per cent. The plasma glucose level was maintained by a constant intravenous infusion delivered by a calibrated pump. The sustaining infusion for the diabetic patients contained 15 to 25 per cent glucose and was delivered at 3 ml. per minute. Twenty minutes were allowed for equilibration between the start of sustaining infusion and the beginning of the ten minute urine collections.

In the insulin experiments, regular Lilly or Squibb preparations were used. Twenty units were given intravenously as a priming dose while 2 units per minute were delivered in the sustaining infusion. Again, 20 minutes were allowed for equilibration before the start of the ten minute collection periods.

Inulin clearance was used as a measure of glomerular filtration rate. The plasma inulin level was maintained between 30 and 40 mgm. per cent. All urines were collected through a multi-eyed catheter. The bladder was washed out at the end of each period except when the urine flow exceeded 10 ml. per minute. Arterial bloods were obtained at the mid point of each period.

Inulin was determined by the Harrison modification (6) of the Alving, Rubin and Miller method (7). Prior to the inulin measurement, glucose was removed from each 1 ml. sample of plasma and diluted urine by exposure for 30 minutes to 6 ml. of a 30 per cent yeast suspension. Glucose was determined by a modification (4) of the Folin method. Glycolysis was inhibited by the addition of a small amount of fluoride to each blood sample, which was centrifuged immediately. The plasma was drawn off within 15 minutes of collection.

Plasma Glucose Levels and the Ratios of Glucose Load to Tm_G

The ranges of arterial plasma glucose levels achieved during the measurement of Tm_G alone and of Tm_G during the administration of insulin are recorded in Table II. Generally the plasma glucose level was between 500 and 800 mgm. per cent and rarely varied more than 50 mgm. per cent during the measurement of Tm_G alone. Following the administration of insulin the plasma glucose level with one exception declined in spite of the continued glucose infusion. The fall in plasma glucose during the measurement of Tm_G while insulin was being administered averaged 57 mgm. per cent with a range of 0 to 121 mgm. per cent. A glucose load to Tm_G ratio of 1.5 or higher is generally accepted (4) as being adequate to completely saturate the normal tubular reabsorptive capacity for glucose. Ratios exceeding this value were achieved in all but two of the diabetic patients and in all but one of the non-diabetic subjects. The lowest ratio of 1.25 occurred in a patient with an unusually high Tm_G. Any error introduced by a too low load of glucose would result in a falsely low Tm_G. Because of the reduction in Tm_G resulting from insulin administration, the glucose load to Tm_G ratio was maintained after insulin, even though the load decreased because of the falling plasma glucose level.

TABLE II

Plasma glucose levels and ratios of glucose load to Tm_G

All plasma glucose levels done on arterial blood. Initial and final plasma glucose levels are recorded for the three periods that made up each observation, except those marked* which consisted of five periods and those marked† which consisted of four ten minute periods.

Patient	Plasma glucose, *mgm. per cent*		Ratio glucose load to Tm_G	
	Tm_G alone	Tm_G +Insulin	Tm_G alone	Tm_G +Insulin
1	645–735	703–675	2.01	2.48
2	450–420	420–402	1.34	1.33
3	554–616	616–546	1.54	1.69
4	567–606	548–533	1.75	1.93
5	470–480	460–440	1.25	1.24
6	690–721	683–613	2.12	1.92
7	605–640	640–550	1.71	1.91
8	438–425	425–348	1.43	1.58
9	624–636*	610–550*	1.77	1.76
10	624–654*	666–545*	1.62	1.65
11	765–840*	840–780*	2.30	2.59
12	885–900	930–870	2.55	2.06
		Average	1.64	1.70
13	910–990	—	2.56	—
14	590–600	620–510	2.52	2.15
15	1022–1035*	1404–1368*	3.44	4.38
16	585–600†	585–585†	1.77	2.02
17	504–500	—	1.78	—
18	812–798†	770–693	2.98	2.68
19	580–630	—	1.72	—
20	410–460	—	1.44	—
21	660–717	—	2.34	—
		Average	2.28	

RESULTS

The values for glomerular filtration rate (GFR), Tm_G and the ratio of GFR to Tm_G observed in 12 diabetic and in nine normal subjects are recorded in Table III along with normal values established by Smith (8). GFR and Tm_G values are corrected to a surface area of 1.73 sq. m.

The group of normal subjects is not large, but their filtration rates and Tm_Gs are in the normal

range established by Smith and his associates (8), with the exception of one Tm_G (patient 20, Table III) which exceeded the mean normal plus 2 standard deviations ($375 + 2 \times 79.7 = 543$ mgm. per minute for males, $303 + 2 \times 55.3 = 414$ mgm. per minute for females).

The diabetic group is also small and, although the mean Tm_G for both males and females are greater than the corresponding normal values, the differences are not statistically significant. However, Tm_G exceeded the mean normal plus 2 standard deviations in five of the 12 diabetic patients. Application of the binomial theorem indicates that the possibility of this occurring by chance alone is remote. Although patients with hypertension or those who had frank renal disease were excluded from the study, two of the female patients with low filtration rates (patients 8 and 12) were the only diabetics to have Tm_Gs less than the mean normal value.[2]

[2] Three diabetic patients with frank renal disease were also studied but excluded from this report. These patients had glomerular filtration rates of 39, 45, and 70 ml. per minute, Tm_Gs of 109, 131, and 183 mgm. per minute, and GFR/Tm_G ratios of 0.36, 0.34, and 0.38 respectively. The first two patients had chronic glomerulonephritis, the last one hypertensive renal disease.

TABLE III

Glomerular filtration rate, Tm_G and ratio of filtration rate to Tm_G in non-diabetic and diabetic subjects

Glomerular filtration rate (GFR) and Tm_G values are corrected to a surface area of 1.73 sq. m.

Group	Patient	Glomerular filtration rate (*ml. per min.*)	Tm_G (*mgm. per min.*)	GFR/Tm_G
Normal Male	Smith (8)	131, ±21.5	375, ±79.7	0.371, ±0.0563
	13	132	521	0.25
	14	133	306	0.44
	15	125	372	0.34
	16	148	470	0.32
	17	125	354	0.35
	18	146	402	0.36
	Average	135	404	0.34
Normal Female	Smith (8)	117, ±15.6	303, ±55.3	0.395, ±0.0617
	19	117	414	0.28
	20	143	422	0.34
	21	111	322	0.34
		—	—	—
Diabetic Male	1	120	415	0.29
	2	143	514	0.28
	3	104	402	0.26
	4	112	377	0.30
	5	164	619	0.26
	6	138	450	0.31
	Average	130	463	0.28
Diabetic Female	7	120	438	0.27
	8	91	271	0.34
	9	117	419	0.28
	10	118	461	0.26
	11	140	495	0.28
	12	65	226	0.29
	Average	103	356	0.30

TABLE IV

Effect of insulin on Tm_G and the ratio of glomerular filtration rate (GFR) to Tm_G

Group	Patient	Tm_G, *mgm. per min.*			GFR/Tm_G		
		Control	Insulin	Δ	Control	Insulin	Δ
Diabetic	1	415	352	−63	0.29	0.37	+0.08
	2	514	496	−18	0.28	0.32	+0.04
	3	402	361	−41	0.26	0.29	+0.03
	4	377	314	−63	0.30	0.36	+0.06
	5	619	539	−80	0.26	0.28	+0.02
	6	450	427	−23	0.31	0.29	−0.02
	7	438	394	−44	0.27	0.32	+0.05
	8	271	197	−74	0.34	0.41	+0.07
	9	419	401	−18	0.28	0.30	+0.02
	10	461	420	−41	0.26	0.27	+0.01
	11	495	445	−50	0.28	0.31	+0.03
	12	226	204	−22	0.29	0.22	−0.27
	Average	424	379	−45	0.27	0.31	+0.04
Non-Diabetic	13	330	216	−114	0.44	0.57	+0.13
	14	372	396	+24	0.34	0.32	−0.02
	15	470	416	−54	0.32	0.35	+0.03
	16	402	404	+2	0.36	0.36	−0.00

Normally glomerular and tubular functions are closely correlated and the ratio of glomerular filtration rate to Tm_G is perhaps the most desirable means for comparison of Tm_G among different groups of subjects. The average GFR/Tm_G ratios of the normal male and female subjects shown in Table III do not differ significantly from Smith's normal series. The ratios for the diabetic patients, however, are low. When the diabetics are compared to Smith's normal group, the t values for the critical ratios are 5 for the males and 4 for females. These values are indicative of a highly significant increase of Tm_G in relation to the glomerular filtration rate among the diabetics as compared to normals.

The difference between the male diabetic group and the normal group recorded in Table IV is barely significant, and the difference between the female diabetics and the three normal females is not significant. However, the normal groups in Table IV are so small that the comparison with Smith's larger series of normal subjects appears to be the more valid. The measurements of this study were made by techniques identical with those of Smith (8).

The filtration rates used to establish the GFR/Tm_G ratios discussed above were those observed during the Tm_G measurements. In five normal subjects and in 10 diabetics, glomerular filtration

rate was measured before the start of the glucose infusions necessary for the Tm_G observations. The filtration rate increased 1 ml. per minute on the average in the non-diabetic group, and decreased 7 ml. per minute in the diabetic group.

The administration of insulin reduced the Tm_G acutely in all 12 diabetic subjects studied (Table IV). The decrease ranged from 18 to 20 mgm. per minute with an average of 45 mgm. per minute. This represented an 11 per cent decrease. The ratio of GFR/Tm_G increased in 10 of the 12 subjects. This was mainly the result of changes in Tm_G although slight increases in filtration rate contributed to the effect in several patients. The latter decreased only 1 ml. per minute on the average during the insulin periods as compared to those obtained during the measurement of Tm_G without insulin. A progressive fall in filtration rate with no obvious explanation was noted throughout the study in one of the exceptions (patient 12). Insulin was also administered to four non-diabetic subjects (Table IV). A decrease in Tm_G occurred in two of these subjects.

Tm_G was measured in one diabetic patient during seven consecutive periods without the administration of insulin. The total length of this study was equal to the longest study in which insulin was administered. Throughout this control study the GFR/Tm_G ratio was constant and the difference between the lowest and the greatest Tm_G values was 26 mgm. per minute.

DISCUSSION

Five of 12 patients with mild to moderately severe diabetes, uncomplicated by known renal disease, were found to have Tm_G values greater than the accepted normal range.[3] Tm_G was above the mean normal in 10 of the patients. The two exceptions had decreased glomerular filtration rates although hypertension, proteinuria or other evidences of renal disease were absent. The reduction in renal functions in these patients may have been due to arteriosclerosis of the kidneys, or perhaps represented the early stages of intercapillary glomerulosclerosis.

[3] Richardson (9), working independently, has found the Tm_G to be greater than the normal range in six of 14 diabetic patients.

The ratio of filtration rate to Tm_G was significantly reduced among the diabetic patients. This appeared to be the result of increased Tm_G values rather than of decreased glomerular filtration rates, for in the majority of patients the filtration rates were in the normal range. In addition, the low filtration rate to Tm_G ratios were not due to possible alterations in glomerular activity induced by the glucose infusions.

The administration of insulin decreased the Tm_G in each of 12 diabetics tested and increased the GFR/Tm_G ratio toward normal in 10 of the 12. Insulin decreased the Tm_G in two of four non-diabetic subjects. Although slight decreases in plasma glucose levels usually occurred after insulin administration, this was kept at a minimum by the sustaining infusion of glucose. In any case, adequate glucose load to Tm ratios were maintained throughout the studies.

The decreased Tm_G after insulin was not due to increased utilization of glucose by the renal tubules for this would be reflected by an *apparent increase* in Tm_G. Whether the effect of insulin on the Tm_G was due to insulin itself or to a contaminant such as the "hyperglycemic factor" (10) is not certain. However, in the present experiments and in the doses used, insulin given intravenously did not raise the blood glucose values.

An increase of Tm_G in diabetes might be attributed to insulin deficiency itself or might be secondary to other hormonal imbalances that may play a role in diabetes. The reduction of Tm_G effected by insulin is not readily explicable on the basis of the usual concepts of insulin action. Offhand, a greater ability of the renal tubule cells to transport glucose would be expected. But in any case, the decreased Tm_G after insulin may explain clinical observations in diabetics that are reported from time to time such as: "after prolonged insulin therapy the urine may contain sugar even though the blood sugar is much lower than before treatment and the urine may have been negative before insulin therapy was instituted" (1).

Since insulin decreases Tm_G in diabetics, it is possible that the differences between the GFR/Tm_G ratios of diabetics and non-diabetics may be the result of increased secretion of endogenous insulin in the non-diabetic occasioned by the rather large loads of glucose. This endogenous insulin

would lower the Tm_G perhaps maximally and additional exogenous insulin would thus have little or no effect. In line with this hypothesis was the failure of insulin to reduce Tm_G in two of the four normal subjects tested. In the diabetics the glucose load does not call forth as much insulin, and exogenous insulin would be free to exert its ability to reduce Tm_G. In view of the equilibration period allowed to achieve relatively stable plasma glucose levels, this point could not be critically examined. However, a downward trend in Tm_G was not apparent in successive periods among the non-diabetic subjects.

CONCLUSIONS

1. The maximum capacity of the renal tubules to reabsorb glucose is above the normal range in a significant proportion of patients with mild to moderately severe diabetes.

2. When related to the glomerular filtration rate, the glucose reabsorptive capacity is increased in diabetics without renal disease.

3. Insulin reduces the glucose reabsorptive capacity of diabetics.

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THE HAEMOLYTIC FACTOR IN THE ANAEMIA OF LYMPHATIC LEUKAEMIA[1]

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(Submitted for publication June 24, 1950; accepted, November 6, 1950)

Cases of lymphatic leukaemia complicated by a haemolytic anaemia have been reported by Haden (1), Singer and Dameshek (2), Wiseman (3), Feldman and Yarvis (4) and by Stats, Rosenthal and Wasserman (5) among others. It has been the commonly held view that such cases are rare and that the cause of the anaemia usually found in association with lymphatic leukaemia lies in a disturbance of erythropoiesis the result of replacement of erythropoietic tissue by leukaemic tissue. That haemorrhage is also an etiological factor in the anaemia in some cases is clear to everyone, but that excessive haemolysis may also be a factor is by most authors considered an improbability. Because of the haemosiderosis which they found and the hyperplasia of erythropoietic tissue which was demonstrated in some of their cases, earlier workers (6–8) believed that excessive haemolysis probably played an important role. This view was disputed by Forkner (9) who cited the work of Whipple and Robscheit-Robbins (10) which showed that there was only a little more iron in leukaemic than in normal livers. Recently the problem has been reviewed by Collins and Rose (11) who admit that excessive haemolysis may be present but state that in the case of lymphatic leukaemia the anaemia is due in most cases to depression of erythropoiesis.

It has been the purpose of the present investigation to determine by means of the differential transfusions of Ashby whether or not excessive haemolysis is an important factor in the production of the anaemia of lymphatic leukaemia even when the usual signs of excessive haemolysis are absent and whether in any cases there is evidence of the presence of a haemolytic process not seen in normal subjects.

[1] This work was supported by grants-in-aid from the Medical Division of the National Research Council.

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EXPERIMENTAL METHOD

The cases studied were transfused with 500–1,500 cc. of stored whole blood. The blood had been stored for one to four days in the proportion of 400 cc. of blood to 150 cc. of an anticoagulant mixture containing 2.3 gm. dextrose U.S.P. and 1.7 gm. sodium citrate U.S.P. per 100 cc. The inagglutinable erythrocytes were counted the day after transfusion, then every other day for 10 days and subsequently at intervals of 10 to 14 days. The method of differential agglutination was that described by Dacie and Mollison (12). With this method it has been found that the standard error of the difference between duplicate observations is of the order of 6% of the inagglutinable count (13). In the present study four counts were made on each specimen, and in some cases two counts by two observers. The results have been expressed numerically in the manner described by Brown and associates (13) who worked on the hypothesis that in some cases of anaemia two processes of erythrocyte destruction could be distinguished and who named these the *linear haemolytic mechanism* and the *exponential haemolytic mechanism*. This method of description provides *1*) an estimate of the average life of the transfused erythrocytes ($\bar{t}$), *2*) the fraction of the transfused erythrocytes which has been destroyed by the *exponential haemolytic mechanism* (F_e) and *3*) a fictitious average life which would have obtained if the linear haemolytic mechanism had been acting alone ($\bar{t}_e$). These are derived from the equation.

$$\frac{N}{N_o} = (1 - L_e t)\,\frac{R \exp(-L_e t) + 1}{R + 1}$$ [4]

which has been found to be the simplest equation which reasonably fits the data in those cases where the plotted results show considerable curvature. When the decay curve is linear the equation becomes

$$N = N_o(1 - L_e t)$$

It has been found by Callendar and colleagues (14) that

[4] Notation

- N = Donor cell count in recipient at time t, million per c.mm.
- N_o = Donor cell count in recipient immediately after transfusion, million per c.mm.
- t = Time measured from transfusion (days)
- L_e, L_e, R = Constants.
- $\bar{t}$ = Average life of transfused cells measured from t = 0 (days)
- $\bar{t}_e$ = Average life corresponding to linear component (days)

in a normal recipient the decay curve is linear and that the average life of the transfused erythrocytes is about 60 days. This corresponds well with the estimates of life span of the erythrocyte which have been made by those who fed a normal subject glycine containing N^{15} (15) and by those who have used pigment excretion studies as the basis for estimation (16).

The cases which are reported now were not selected except with respect to suitability of blood group and accessibility during the period of observation.

RESULTS

Case I, J. H. (48–3506), a man 67 years of age had been a known diabetic for 10 years. For one year before admission he had complained of dyspnoea and substernal distress on exertion and latterly of nocturnal dyspnoea. Examination on admission showed moderate enlargement of the the cervical lymph nodes, enlargement of the liver, a heart which was borderline in size, rales over the lower half of both lung fields, and fluid in the right pleural cavity. Urinalysis—trace of albumen, varying glycosuria. HGb. 5.8 gm.%. R.B.C. 1,740,000. Haematocrit 18%. W.B.C. 650 with 58% lymphocytes, 16% prolymphocytes, 4% lymphoblasts, 20% polymorphonuclear neutrophiles and 2% band cells. Reticulocytes 2.4%. Platelets 31,680. Serum Bilirubin 0.4 mg.%. Fasting Blood Sugar 231 mg.%. Blood Urea 33 mg.%. Serum Chlorides 586 mg.%. Serum Proteins 6.3 gm.%. with 4.12 gm. albumin and 2.18 gm. globulin. Plasma Prothrombin Content 62%. Faecal Urobilinogen 631 mg./day during a three-day collection by the method of Schwartz, Sborov and Watson (17). Bone marrow smear showed 6% small lymphocytes, 25.5% large lymphocytes, 14.5% prolymphocytes, 42% lymphoblasts and an L/E ratio of 9/1. Chest x-ray showed a pneumonic infiltration of both lung roots and increased markings in the upper half of the left lung. C/T ratio of 16.5/30. Electrocardiogram—chronic myocardial pathology. He was treated with rest, digitalis, insulin, a low

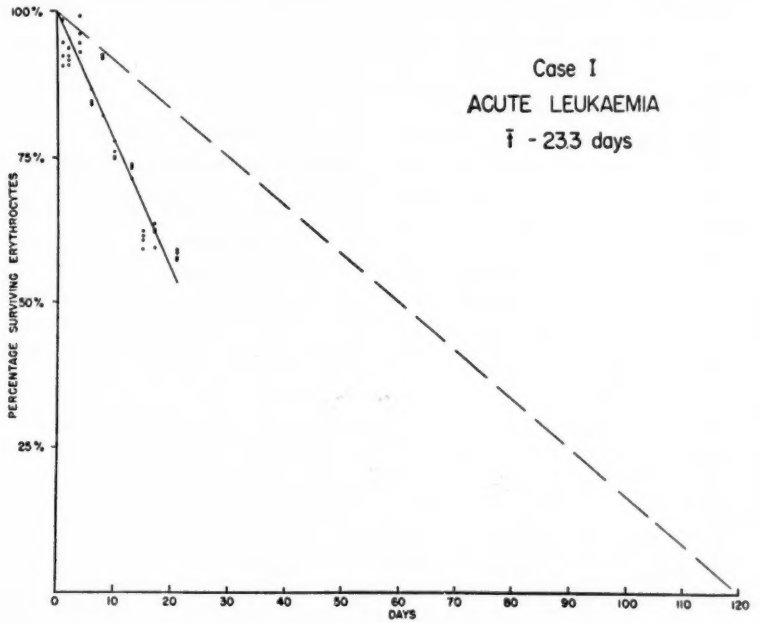

FIG. 1. SURVIVAL OF TRANSFUSED ERYTHROCYTES IN CASE I

The dots represent the actual observations and the solid line has been drawn through points calculated from the fitted quotation. The broken line represents the decay curve in a normal subject.

sodium neutral ash diet, and Fowler's solution. The diabetes mellitus was brought under good control. He died at home eight weeks after his admission to hospital.

The test transfusion was followed for 21 days. When the number of surviving erythrocytes was plotted against time the decay curve was found to be linear for that period (Figure 1). It showed an increased slope as compared with that found in normal subjects (14) and the calculated average life of the transfused cells ($\bar{t}$) was 23.3 days.

Case II, W. H. (27913), a man aged 54 had been suffering from disseminated sclerosis for 13 years. For six years he had been aware of swellings in his axillae and groins and for one year there had been a mass in his abdomen. He also complained of pallor, increasing dyspnoea on exertion and of oedema of the ankles. Examination showed a pale wasted man with generalized lymphadenopathy, a palpable liver and a greatly enlarged spleen. There was the characteristic evidence of moderately advanced disseminated sclerosis but for two years there had been no increase in the disability from this cause. Urinalysis was negative. HGb. 4.4 gm.%. R.B.C. 1,850,000. Haematocrit 17%. W.B.C. 1,050,000 with 94% lymphocytes. Reticulocytes 1.0%. Platelets 60,000. Serum Bilirubin less than 0.2 mg.%. He lived for about 18 months following this admission to hospital.

The test transfusion was followed for 38 days and the plotted results showed considerable curvature (Figure 2). The calculated average life of the transfused cells ($\bar{t}$) was 18 days and the proportion destroyed by the *exponential haemolytic mechanism* (F_e) was 0.34.

Case III, C. W. (49–4189), a man aged 68 had noted loss of strength and lumps in his neck for one year. There were enlarged lymph nodes to be palpated in the cervical, occipital, axillary, epitrochlear, inguinal and popliteal regions. The liver edge was just palpable and the spleen was enlarged to below the level of the umbilicus. Urinalysis negative. HGb. 9.1 gm.%. R.B.C. 3,200,000

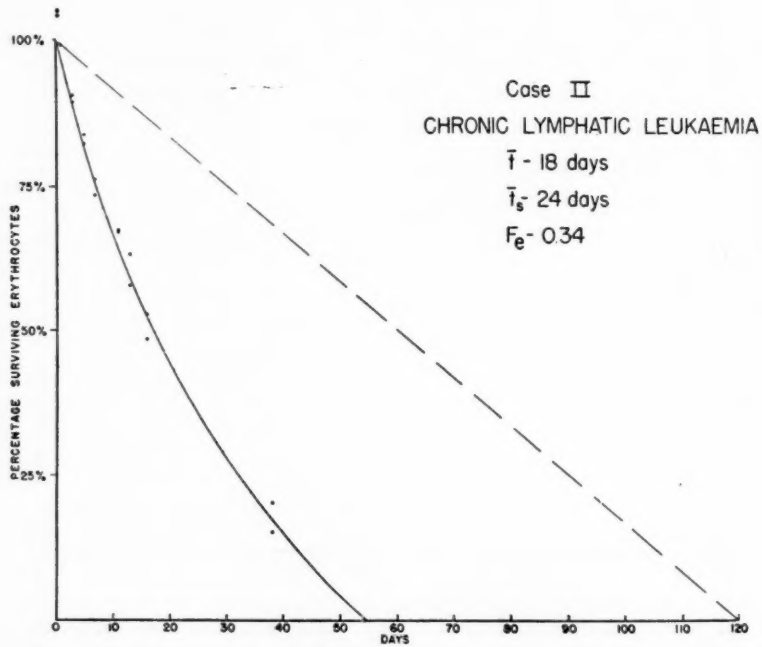

FIG. 2. SURVIVAL OF TRANSFUSED ERYTHROCYTES IN CASE II

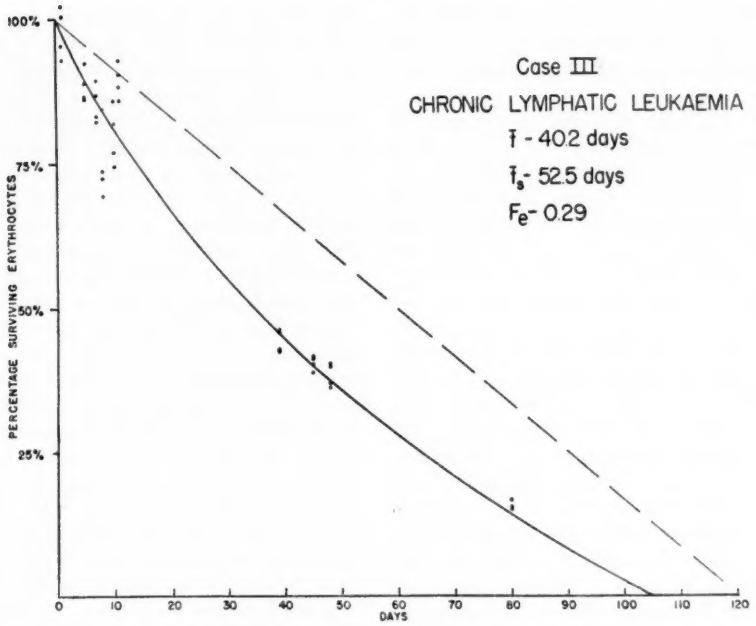

FIG. 3. SURVIVAL OF TRANSFUSED ERYTHROCYTES IN CASE III

Haematocrit 29%. W.B.C. 29,800 with 96% lymphocytes, 2% polymorphonuclear neutrophiles, 1% band cells, 1% myelocytes. Reticulocytes 1.1%. Platelets 169,000. Serum Bilirubin 0.4 mg.%. Faecal urobilinogen 31.9 mg./day. Lymph node biopsy showed the characteristic picture of lymphatic leukaemia. He was given deep x-ray therapy amounting to 300r over both supraclavicular and both axillary regions during a period four to eight days following his test transfusion. Radiotherapy was then discontinued because his white blood count had fallen to 10,000. He received further radiotherapy six weeks later. He is still alive, a year after the test transfusion.

The plotted results in this case showed an increased slope and also some curvature (Figure 3). The fraction of the transfused cells which

TABLE I

Blood counts following the subcutaneous injection of 1 cc. 1/1,000 adrenalin in Case IV

	Before	5 minutes	10 minutes	20 minutes	35 minutes
HGb., gm. %	10.4	10.4	10.6	10.7	10.6
R.B.C.	3,320,000	3,230,000	3,420,000	3,480,000	3,250,000
W.B.C.	187,000	355,000	299,000	298,000	342,000
Lymphocytes, %	97		81	75	77
Smudge cells, %			18	21	15
Lymphoblasts, %				1	1
Monocytes, %	1				
Polymorphs. %	2		3	3	7
Platelets	172,600	329,400	1,585,000	174,000	435,500

had been destroyed by the *exponential haemolytic mechanism* (F_e) was estimated to be 0.29 and the average life of the transfused cells ($\bar{t}$) was 40.2 days.

Case IV, F. E. B. (49–9730), a man aged 68 gave a history of fatigue and weakness for three years and increasing dyspnoea on exertion for one year. For a month he had had a productive cough. He was pale and emaciated and showed a generalized lymphadenopathy and moderate enlargement of the spleen. There was marked arteriosclerosis of the Monckeberg type and his heart was grossly enlarged. There were numerous coarse rales to be heard over the lower lobes of both lungs. Urinalysis negative. HGb. 6.95 gm.%. R.B.C. 2,340,000. Haematocrit 22%. W.B.C. 330,000 with 97% lymphocytes, 2% polymorphonuclear neutrophiles, 1% eosinophiles. Reticulocytes 1.4%. Platelets 730,080. Blood counts following the injection of 1 cc. 1/1000 adrenalin are shown in Table I. Serum Bilirubin 0.5 mg.%. Faecal Urobilinogen 56.8 mg./day. Serum N.P.N. 48 mg.%. Plasma Protein 6.49 gm.% with 3.96 gm. albumin and 2.53 gm. globulin. Sputum contained large numbers of lymphocytes, some of them lying in sheets. Chest x-rays showed persistent diffuse linear streaking and some mottling in the lower halves of both lung fields. Two sternal punctures yielded no marrow. Treatment with urethane was begun nine days after his test transfusion and in the next three weeks there was considerable improvement with a gain in weight, an increase in haemoglobin from the post-transfusion level of 11 gm.% to 12.1 gm.% and a fall in white blood count to 182,000. The improvement continued with a daily dose of 4.5 gm. urethane so that after three months his white blood count had fallen to 23,000. His haemoglobin at that time was 11.1 gm.%. His lung fields were clear on x-ray.

The result of the transfusion in this case was entirely within normal limits. The decay curve was strictly linear and interrupted the base line at 114 days.

DISCUSSION

To say that evidence of an increased rate of destruction of transfused erythrocytes is evidence of an increased destruction of the patient's own cells is to assume that the two are treated by the body in the same way. That this is not always the case has been shown in congenital haemolytic icterus (18), nocturnal haemoglobinuria (12) and sickle-cell anaemia (19). In these conditions transfused cells from a normal donor may survive for a normal length of time, though it is obvious that the patient's own cells are being destroyed abnormally rapidly. In these three conditions, the reverse also holds, and the patient's cells when transfused into a normal recipient are destroyed more rapidly than the cells of a normal donor. Save in these conditions there is no evidence that cells from a normal donor are destroyed in the recipient faster than the recipient's own cells provided there are not present in the recipient antibodies against the donor cells (mis-matched transfusion) and also, possibly, provided the total erythrocyte count is not raised by transfusion above the recipient's normal count. Such evidence would be admittedly difficult to obtain, and the point of identical treatment of the donor's and the recipient's cells, with the exceptions already mentioned, cannot be proven. It can be said, however, that in those cases in this and in previous work (13, 20) where there was the conventional evidence of excessive haemolysis, *viz.* reticulocytosis, and increase in serum bilirubin and faecal urobilinogen, the average life of the transfused cells has always been markedly diminished. It can also be pointed out that the inferences to be drawn from the present work involving the transfusion of cells from normal donors into abnormal recipients, followed on a comparison of the results of work involving the transfusion of cells from normal donors into normal recipients (14).

The suggestion has been made that in patients with enlarged spleens, some of the transfused erythrocytes may be "lost" in the spleen giving a falsely low survival time, but there seems no reason to suppose that the transfused erythrocytes are more likely to be contained in the spleen than are the patient's own cells. That both may disappear from the peripheral circulation into the spleen is obvious. If the transfused erythrocytes were indiscriminately "lost" in the splenic pulp, an increase in the exponential component of the decay curve would follow and there would

be no increase in the activity of the *linear haemolytic mechanism* if indiscriminate "loss" in the spleen were the only abnormal process at work. Data which would obtain in this situation have not been found in this or earlier series (13, 20). Abnormally rapid removal of the transfused erythrocytes by the spleen from the main mass of circulating erythrocytes according to some characteristic of the erythrocytes themselves, such as age, would result in a linear decay curve with increased slope. Any significant return of these cells to the systemic circulation would cause upward deviations of the decay curve which have not yet been observed. In summary rebuttal of the suggestion that an enlarged spleen may vitiate the results in the present experiments, it may be said that there is no reason to suppose the transfused erythrocytes are removed from the circulation more rapidly by the spleen than are the patients' own cells and that if both are removed abnormally rapidly there is no evidence to date that they are returned to the circulation in significant numbers.

In three of the four cases of lymphatic leukaemia which have been studied, the transfused erythrocytes were destroyed at a rate faster than normal, which is interpreted as evidence of excessive haemolysis in these cases. What part, if any, the urethane therapy had in the results of Case IV, it is impossible to say. During the period of observation his haemoglobin was maintained at the immediate post-transfusion level and there was a marked improvement in his general condition, with diminution in size of his spleen and accessible lymph nodes. He was, in fact, in a remission. In Cases II and III the reticulocyte count and the serum bilirubin, and in Case III the faecal urobilinogen, were within normal limits, despite the definite increase in rate of destruction of transfused erythrocytes. This suggestion that the present experimental method is a more sensitive indicator of excessive haemolysis than those more commonly employed has been presented before (20). In Cases II and III there was also evidence of activity of the *exponential haemolytic mechanism*. The *exponential haemolytic mechanism* has not been seen in normal recipients except when the post-transfusion erythrocyte count has been above normal and it has not been evident in this or previous series except when there has also been increased activity of the *linear haemolytic mechanism*. In Case I, the acute case, where the observed portion of the decay curve was sensibly linear, it should be remembered that the period of observation was only 21 days and that further counts might have provided evidence of the *exponential haemolytic mechanism*.

TABLE II

Survival of transfused erythrocytes in four cases of lymphatic leukaemia

Case No.	Average life ($\bar{t}$ days)	Average life corresponding to linear mechanism ($\bar{t}_a$ days)	Fraction destroyed by exponential mechanism (F_e)
I	23.3	23.3	0
II	18	24	0.34
III	40.2	52.5	0.29
IV	57	57	0

These results would seem to show that in at least some cases of lymphatic leukaemia the anaemia is contributed to by increased haemolysis and that in some cases there is evidence not only of increased haemolysis but also of haemolysis of an abnormal type. It is not necessary in three of the present four cases to explain the anaemia entirely on the basis of diminished erythropoietic activity, and indeed in Case II where the rate of haemolysis was more than three times that of normal, the haemolytic activity was of an order which might entirely explain the anaemia found. The fact that excessive haemolysis has been demonstrated in cases where the more usual indices of haemolysis have been lacking suggests that it may be an important factor more often than is now believed. In what proportion of cases this holds, remains to be determined by further work. The physiological mechanism and the sites of the different types of haemolysis which have been observed also remain speculative. The question of a relation between the two types of haemolytic activity which have been demonstrated here and the activity of immune bodies which have been demonstrated in some cases of haemolytic anaemia in leukaemia (21) will have to be answered by further work.

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THE PRECIPITABLE IODINE OF SERUM IN NORMAL PREGNANCY AND ITS RELATION TO ABORTIONS[1]

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(Submitted for publication August 2, 1950; accepted, November 6, 1950)

In 1948 it was reported by Heinemann, Johnson and Man (1) that early in pregnancy the precipitable (protein-bound) iodine in the serum (SPI) rose to concentrations in the upper range of normal or even to levels that outside of pregnancy are characteristic of hyperthyroidism. It also appeared that if the SPI did not rise, the pregnancy was likely to end in abortion within the first four months. Some evidence was presented that such accidents might be prevented by the administration of active thyroid substance. The present paper deals with a further investigation of the phenomenon.

MATERIAL

Patients from the Clinics of the Department of Obstetrics and Gynecology in the New Haven Hospital form part of the material studied. In addition, analyses of the serum of patients have been made for members of the Clinical Faculty who have permitted the data to be used and have generously contributed the clinical information required for the purposes of this investigation. Finally, a certain number of patients with disorders of function of the thyroid were drawn from the Metabolism Clinic.

In many cases only single observations are available. In some, determinations of SPI were made at intervals throughout pregnancy. In a limited number observations were continued into the early postpartum period. For obvious reasons, figures preceding pregnancy were only occasionally obtained. In a certain proportion of cases serum lipids were also measured.

METHODS

Venous blood was drawn, with precautions against contamination by iodine, from patients in the postabsorptive state or after a meal free from fat (fruit juice, black coffee, and toast, either dry or with jam). Serum was removed under anaerobic precautions. Lipids were measured by the method of Man and her associates (2-6). Until July, 1949, SPI was measured by the method of Riggs and Man (7); after that date by a micromodification (8). Analytical results by the two methods agree.

RESULTS

Normal pregnancy. In Figure 1 are presented the values for SPI obtained on one or more occasions from 46 normal women during the course of pregnancies that proceeded to term without complications. None of these patients received thyroid or any medication known to affect thyroid function during their pregnancies or in the periods immediately preceding or following pregnancy. Four had goiters without evidence of abnormal activity of the thyroid. Of the 56 determinations from the end of 16 weeks until term, SPI was only twice lower than 5.6 γ per cent, and in all but three instances was 6.0 γ per cent or higher. Values lower than 6.0 γ per cent were only transitory incidents. The average concentration of SPI in nonpregnant normal persons is 5.0 γ per cent (8). In the last 24 weeks of normal pregnancy, therefore, SPI was invariably above the average normal value for nonpregnant persons. It is not uncommon to find values up to 10 γ per cent which, outside of pregnancy, would denote severe hyperthyroidism. In pregnancy, however, such concentrations are associated with no symptoms, signs, nor metabolic disorders indicative of overactivity of the thyroid gland.

In the same figure is illustrated the course of SPI in the progress of pregnancy in a series of patients in whom repeated observations were made. There is no definite tendency for SPI to rise or fall during the last 24 weeks. It is, however, subject to fluctuations far greater than those that have been observed in nonpregnant individuals. Variations of 2 γ per cent over relatively short intervals are frequent and variations of as much as 4 γ per cent have been encountered, with-

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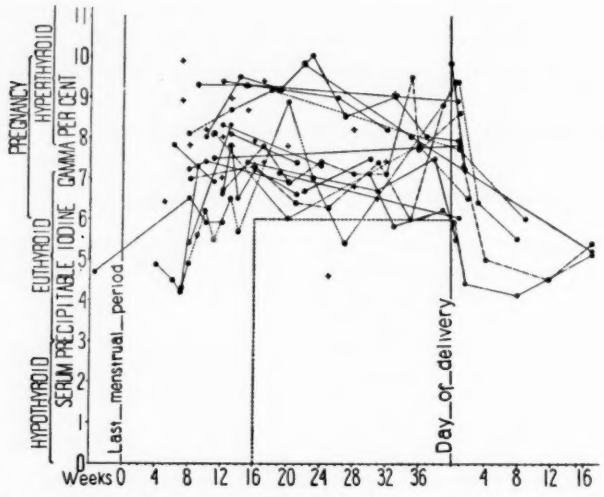

FIG. 1. SERUM PRECIPITABLE IODINE (SPI) IN THE COURSE OF NORMAL PREGNANCY

Crosses represent single observations. Circles connected by lines represent repeated observations.

out any discernible disturbances. In normal individuals SPI is quite stable (8, 9).

In many or most instances the SPI rises sharply at or near the onset of pregnancy. Concentrations in the hyperthyroid range have been obtained as early as six weeks after the last menstrual period. In only one case was an observation obtained preceding pregnancy. In this case SPI had risen from 4.7 to 6.5 γ per cent by the time the first measurement in pregnancy was taken, eight weeks after the last mentrual period. In a certain proportion of subjects, however, the rise is distinctly delayed; and in most it continues after the initial sharp increase. The same large fluctuations observed during the later part of pregnancy are evident in these early stages. Sometimes, as a result of these fluctuations, SPI, after the initial rise, drops down again temporarily into the nonpregnant range.

After delivery SPI diminished again, returning to normal nonpregnant values. The consistency of this fall, like that of the initial rise, indicates quite clearly that the elevation of SPI during pregnancy is a feature characteristic of pregnancy, not of the subjects of this particular study. The data are too scanty to permit precise definition of the course of the decline. Occasionally it was quite precipitate. In most instances it did not become evident until the second week postpartum, or even later. At first it was rapid, thereafter continuing, sometimes for a period of several weeks, at a more gradual rate. The possibility that these variations may be related to lactation deserves inquiry. When the decline was delayed, signs of overactivity of the thyroid remained in abeyance. The tolerance to high SPI appears to persist for an appreciable interval after the termination of pregnancy.

In addition to the subjects of Figure 1, 14 apparently normal women are still undelivered at the present date. Table I shows the duration of pregnancy to date in each case, the SPI values and the duration of the pregnancy at the time the SPI was measured. In all cases SPI was greater than 6.0 γ per cent before the end of 16 weeks, usually far earlier. In one instance, IB, after 12 weeks it was 7.9 γ per cent. Though it fell sharply to 4.0 γ per cent at the end of 15 weeks, it had returned to 6.2 γ per cent in another week and thereafter has remained elevated. This illustrates the fluctuations which were mentioned above. When MJ was first seen, after eight

weeks of pregnancy, without any signs of hypothyroidism, her SPI was only 3.7 γ per cent. After ten weeks it had risen only to 4.0 γ per cent. Three weeks later, however, at the end of 13 weeks it had risen to 6.1 γ per cent. After 21 weeks it had dipped slightly to 5.6 γ per cent. Its further course has not been determined, but the patient is within six weeks of term. The woman appears to be one of those with a delayed rise. In two instances SPI was measured before pregnancy. In one, RR, it was above 6.0 γ per cent and had not risen higher eight weeks after the last menstrual period. In the other it had risen from a prepregnancy concentration of 5.7 to 7.4 γ per cent after six weeks of pregnancy. These cases lend support to the thesis that SPI normally rises comparatively early in pregnancy to 6 γ per cent or more and remains elevated until delivery.

TABLE I

Undelivered, apparently normal pregnant women

Patient	Duration of pregnancy*	Weeks after last menstrual period†	SPI
	weeks		*γ per cent*
C5279	38	28	8.6
IB	36	12	7.9
		15	4.0
		16	6.2
		20	6.6
		23	7.0
		27	7.3
		31	7.0
		33	6.9
C38229	19	7	11.9
HC	17	8	7.0
MD	22	8	8.6
VD	24	22	10.6
BH	37	3	7.7
		5	6.0
		7	6.6
		13	9.0
		26	9.0
MJ	34	8	3.7
		10	4.0
		13	6.1
		21	5.6
PJ	16	9	7.7
		14	6.4
MM	26	19	7.1
		25	7.0
Re	37	7	8.1
RR	42	−22	6.3
		8	6.2
CT	27	−29	5.7
		6	7.4
		10	7.8
CeT	20	12	6.4

* The duration at the time the paper was written.
† A minus sign denotes that the observation was made at the indicated interval before conception.

TABLE II

Complicated pregnancies

Patient	Weeks after last menstrual period*	SPI	Complication
		γ per cent	
B73578	20	8.7	Diabetes insipidus
63694	8	9.7	Diabetes mellitus, Kimmelstiel-Wilson
	p 26	7.4	
C2128	40	7.4	Toxemia of pregnancy
C22464	11	7.5	Multiple sclerosis
	p 1	6.6	
86295	17	6.9	Toxemia of pregnancy, thrombophlebitis
C26972	40	6.0	Toxemia of pregnancy
C15296	34	4.8	Toxemia of pregnancy
	40	4.8	
5911	a 4 years	4.1	Diabetes
	40	6.0	
	p 1	4.6	
13258	30	12.1	Toxemia of pregnancy
B77955	8	8.4	Toxemia of pregnancy
B94664	8	7.0	Toxemia of pregnancy
31123	8	7.2	Vascular disease

* a denotes that the observation was made at the indicated interval before pregnancy; p denotes that the observation was made at the indicated interval after delivery.

Abnormal pregnancy. In addition to the patients with normal pregnancies, 12 pregnant women with complications of one kind or another, not involving the thyroid gland, were studied. The observations on these cases are shown in Table II. Of the 12 patients, eight had toxemias of pregnancy or vascular disease, two had diabetes, one with a Kimmelstiel-Wilson syndrome, one had diabetes insipidus and one multiple sclerosis. In spite of these complications, SPI fell in the range characteristic of pregnancy in all but one case, C15296. This patient was studied at the end of 34 weeks and again just before delivery. On both occasions SPI was 4.8 γ per cent. What it may have been earlier or later is unknown. In two instances, 63694 and C22464, pregnancy was terminated: in one for a Kimmelstiel-Wilson syndrome, in the other for multiple sclerosis. In both SPI fell in the characteristic manner after pregnancy. In a patient with diabetes, 5911, SPI was measured before, during, and after pregnancy. The value during pregnancy was 1.9 and 1.4 γ per cent greater than the values before and after pregnancy, respectively. These complications, therefore, appear not to modify the effects of pregnancy upon SPI.

There were two patients with ectopic preg-

nancies. One, three weeks after her last menstrual period, had an SPI of 4.0 γ per cent. Further observations could not be obtained. The second, after ten weeks of pregnancy, had an SPI of 8.3 γ per cent, which had fallen to 6.8 γ per cent 13 days after operation.

Abortions. In Table III are epitomized the records of 26 patients who aborted within the first 16 weeks of pregnancy. SPI was less than 6.0 γ per cent in 18 of the 26. This suggests that failure of SPI to rise during the first 16 weeks is conducive to abortion. Such an inference, however, must be drawn with reservations. In some instances the serum was drawn after bleeding or cramps had begun, occasionally on the day of the abortion. Under such circumstances it is impossible to tell whether the analysis was made before or after the termination of the pregnancy. Sometimes—for example, in Cases DP and MB—the abortion definitely preceded the measurement of

TABLE III

Serum precipitable iodine of patients who aborted

Patient	SPI	Weeks*	Time of abortion† p last SPI	Thyroid‡ given	Remarks
	γ per cent			gm./day	
DB	5.8	12	1 day		
	3.5	p 6			
EBr	4.5	16	0 day		
	5.2	p 22			
EKe	5.6	E	0 day		
B68002	5.4	7	9 weeks		
DP	6.9	13	−? day		Abortion just before SPI
NP	6.5	11			
	5.2	p 52	?		
C5510	7.0	p	−1 day		
ABi	8.3	6	10 weeks	T	
BH	2.7	7			
	4.7	p 8	± 1 day	T	
MG	5.4	8	3 days	Th	
B98775	4.6	15	1 day	Th	
DC	5.8	7		T	May have aborted at time of first SPI
	7.5	10		Th	
	7.1	12	1 week	0.24	
RD	4.0	5		Th	
	7.0	9	4 weeks	0.24	
LJ	4.7	12	2 weeks	Th	
HR	7.6	7		0.12	Slight bleeding
	6.8	12	2 weeks	0.12	
EBe	4.7	6	3 weeks	Th	? Hypothyroid
EI	5.4	10	1 week	0.12	
BK	7.0	7	1 week	0.03	
LL	7.0	7	1 week	0.06	
HS	4.3	E	few days	0.12	
RBa	6.5	a 4			
	5.3	4	few days		
MB	6.1	8	−1 day		
IB	3.3	12	few days	0.18	
VH	3.8	E	few days		
MPo_1	7.3	13		0.33	Abortion threatened
	8.5	16	1 day	0.33	
	4.6	p 4			
MFo_2	2.8	E	9 days	0.12	
	4.4	p 2			
EWi	4.6	a 9			
	5.2	9	4 weeks		
	4.6	p 4			

* If not otherwise marked, indicates weeks after last menstrual period; a indicates weeks before conception; p indicates weeks after delivery. E indicates early in pregnancy, the exact time being unknown.

† A minus sign indicates that SPI was done after the abortion, 0 that it was done on the day of the abortion; ? that the precise interval is unknown.

‡ T indicates that thyroxine was given intravenously immediately after the SPI was done. Th indicates that thyroid was given at the first sign of abortion. The daily dose of thyroid is given when the drug was administered for an appreciable time.

SPI. In other cases, such as B68002 and AB1, the abortion occurred so long after the determination of SPI that it is impossible to relate the two. The SPI of B68002, at the end of seven weeks, was 5.4 γ per cent. It is quite impossible to assert that nine weeks later, when she aborted, it had not risen above 6.0 γ per cent. Conversely, AB1's SPI of 8.3 γ per cent at the end of six weeks may have fallen before her abortion ten weeks later. In five cases (DB, EBr, NP, RBa and EWi) figures for SPI before or after pregnancy are available for comparison. In every case these are distinctly different. In two, DB and EWi, the SPI was higher in pregnancy, but below 6.0 γ per cent. In a third, NP, it was 6.5 γ per cent in pregnancy. In the remaining two, EBr and RBa, SPI appears to have fallen instead of rising during pregnancy. In no instance was the SPI outside of pregnancy below the normal range. In eight cases SPI was over 6.0 γ per cent. Whether or not, therefore, a low value is incompatible with continuation of pregnancy, a high value does not insure against abortion. In seven cases thyroid or thyroxine, or both, were given at the time SPI was measured, because of bleeding, without preventing the abortion. In most of these abortion followed so rapidly that the thyroid can have had little effect. It is possible that some of these patients had aborted before the thyroid or thyroxine was given. DC, for example, was given thyroxine for bleeding after the seventh week. The bleeding stopped and SPI rose and remained high under continued treatment with thyroid. At the end of the 12th week she bled again. The Ascheim-Zondek test at this time proved to be negative. It seems highly probable that she actually aborted on the earlier occasion. RD, on the other hand, did not escape an abortion, despite the administration of enough thyroid to raise her SPI from 4.0 to 7.0 γ per cent. Whether it remained at this level is uncertain, because the dose of thyroid was reduced from 0.24 to 0.12 gm. between the second determination and abortion. Several patients were already receiving variable amounts of thyroid when SPI was first measured. In four of these EI, HS, IB, and MPo_2, SPI was low in spite of the medication. In MPo_2, despite 0.12 gm. of thyroid daily, SPI was lower during pregnancy, 2.8 γ per cent, than it was two weeks after abortion, 4.4 γ per cent. Although these figures suggest that low SPI conduces to abortion, they permit no definite conclusions, chiefly because only single observations are available on subjects who did not receive thyroid. It is clear that administration of thyroid after bleeding has begun will not consistently prevent abortion, even if it raises SPI above 6.0 γ per cent.

TABLE IV

Patients who, having previously aborted (see Table III), had successful pregnancies

Patient	SPI	Weeks*	Thyroid given†	
			Before	After
	γ per cent		gm./day	gm./day
BH	6.5	8	0	0
	5.9	10	0	0
	5.9	12	0	0
MG	8.7	13	0	0
RBa	6.1	4		
	5.8	15	0.09	0.09
MB	5.9	6		0.12
	5.9	10	0.12	0.18
	8.0	11	0.18	0.18
	7.1	18	0.18	0.12
IB	3.8	26	0.15	0.15
MPo	8.3	10	0.24	0.30
	10.0	16	0.30	0.30
	8.6	31	0.30	0.30

* Weeks after last menstrual period.

† *Before* indicates that the patient was receiving thyroid before the determination of SPI. *After* indicates that medication was begun after the determination of SPI.

In Table IV are presented the data from six patients who, having aborted in one pregnancy, successfully carried through another to delivery. The records during the pregnancy that ended in abortion have been given in Table III. The first two, BH and MG, had no complications nor special therapy during their successful pregnancies. Both had, however, far higher SPI's on the second occasions. The SPI of BH after seven weeks of her earlier pregnancy was 2.7 γ per cent; in her successful pregnancy it was 6.5 γ per cent at the end of eight weeks and subsequent figures after ten and 12 weeks were 5.9 γ per cent. MG had SPI's of 5.4 and 8.7 γ per cent, respectively. The comparison in this case is not so precise as it is in BH, because the analysis in the early pregnancy was made after eight weeks, while in the successful pregnancy it was made after 13 weeks. In both instances during the earlier pregnancy, after bleeding had begun, administration of thyroxine and thyroid failed to

prevent abortion. The remaining four cases were treated with thyroid. Among these, RBa and MPo had higher SPI's in their successful pregnancies. The remainder were already receiving thyroid or were given thyroid when SPI was first measured. In every case but one, initial SPI's were greater than 5.0, and subsequent values never lower than 5.8 γ per cent. The exception, IB, is quite unique. The only determination, made after 26 weeks, was 3.8 γ per cent, when the patient was receiving daily 0.15 gm. of thyroid.

Forty-eight women, without evidence of hypothyroidism, successfully completed 51 pregnancies while receiving thyroid substance. This treatment was instituted for such diverse reasons that in the majority of cases it is impossible to determine whether or not it prevented abortions or contributed in any way to the well-being of the mother or fetus. Frequently it was begun before the first observation of SPI or even before the onset of pregnancy because of a history of previous abortions or infertility. So commonly is thyroid administered in pregnancy for various reasons without objective criteria of deficient thyroid function that its beneficial effect is almost impossible to evaluate. In ten of the 57 pregnancies abortion was threatened at one time or another, but pregnancy continued to term. These are presented in Table V. Bleeding or cramps occurred in eight of these during the first 16 weeks. Of these eight only four had SPI's of 6.0 γ per cent or less. In the other four SPI varied from 6.9 to 7.3 γ per cent. Thyroid was given to all eight immediately. Obviously SPI greater than 6.0 γ per cent does not confer protection against bleeding or cramps. But just as obviously it is impossible to assert that these patients would have aborted if thyroid had not been given. In two instances bleeding occurred after 20 and 21 weeks, respectively. In one the SPI had risen gradually from 4.8 γ per cent at seven weeks to 6.6 γ per cent at 20 weeks. The patient had received 0.06 gm. of thyroid daily up to this time, when bleeding began. She was immediately given 2 mg. of thyroxine intravenously. Three days later SPI was 18.1 γ per cent. The bleeding stopped. At the end of four more days when SPI had fallen to 12.8 γ per cent, she was given a continuing dose of 0.12 gm. daily, which was increased to 0.18 gm. at the end of 25 weeks. On this dose SPI fell to 7.8 γ per cent at the end of 33 weeks and to 5.8 γ per cent after delivery. The SPI of the second patient was 7.3 γ per cent at the end of 13 weeks, but six weeks later had dropped to 6.7 γ per cent in spite of the fact that she had meanwhile been given 0.12 gm. of thyroid daily. At the end of 21 weeks she began to bleed. Whether SPI had fallen further was not ascertained. The dose of thyroid was at once increased to 0.24 gm. Six weeks later, when SPI was 8.5 γ per cent, the dose was reduced to 0.12 gm. again because the patient complained of nervousness. With this one exception, possibly transient, at a late stage of pregnancy, after 16 weeks all values were 6.0 γ per cent or higher. In four of the subjects postpartum values were below 6.0 γ per cent, but within the normal range.

TABLE V

Patients with threatened abortions

Patient	SPI	Weeks*	Thyroid given†		Remarks
			Before	After	
	γ per cent		*gm./day*	*gm./day*	
FB	7.3	15	0	0.12	Threatened abortion
	6.4	20	0.12	0.18	
	8.5	30	0.18	0.18	
	4.1	p 9 d.	0‡		
ABu	7.2	12		+	Threatened abortion
B12368	5.6	15	0	0.06	Threatened abortion
	7.4	29	0.09	0.09	
	4.1	p 19	0‡		
CG	5.5	a 11			
	4.8	7	0.06	0.06	
	5.1	11	0.06	0.06	
	6.6	20	0.06	T 2 mg.	Slight bleeding and labor pains
	18.1	20		0.12	3 days later
	12.8	21	0.12	0.18	
	10.0	25	0.18	0.18	
	7.8	33	0.18	0.18	
	5.8	p 15	0.18	0.12	
	5.8	p 28	0.12	0.12	
AL	6.0	5	0	T 1 mg.	Severe cramps
	5.4	12	0.06	0.12	
EM	4.2	6	0	0.18	Slight bleeding
	6.4	13	0.18	0.18	
	6.3	22	0.18	0.18	
	4.5	p 9	0‡		
	5.0	p 30	0‡		
MM (1)	7.3	13	0	0.12	
	6.7	19	0.12	0.12	Spotting at 21 weeks
	8.5	27	0.24	0.12	
	5.8	32	0.12	0.12	
(2)	7.2	11	0.12	0.12	Second pregnancy
PR	6.9	8	0.06	0.06	Bleeding, repeated at 10 weeks
	6.9	12	0.06	0.06	
55146 (1)	5.1	8	0	T 3 mg.	Threatened abortion
	6.8	17	0.12	0.12	
	6.0	38	0.12	0.06	
(2)	5.7	9	0	0	
	6.5	14	0	0.12	
	7.3	18	0.12	0.12	
BT	6.9	11	0	0.12	Bleeding

* a indicates before pregnancy, p indicates after delivery.

† *Before* indicates that the patient was already receiving thyroid. *After* indicates that thyroid was begun after the measurement of SPI.

‡ No thyroid given after delivery.

In these respects, therefore, whether administration of thyroid contributed to the prevention of abortion or not, SPI followed the course characteristic of normal pregnancy.

Effects of administration of dried thyroid or thyroxine. In Table VI are presented the records of 23 women who were given thyroid or larger doses of thyroid specifically because SPI was low in the early stages of pregnancy. All delivered at term. In the majority of those who had repeated examinations, SPI rose above 6.0 γ per cent. The number of prolonged studies is, however, small. In four instances (GM, EWa, EsB and RBl), SPI was less than 6.0 γ per cent between 16 and 20 weeks in spite of thyroid. Two of these probably represent transitory drops: GM to 5.2 γ per cent at 17 weeks, and EWa to 4.7 γ per cent at 18 weeks. The latter had an SPI of 7.7 γ per cent at 29 weeks. In this instance SPI fell from 5.3 γ per cent at 13 weeks to 4.7 γ per cent at 18 weeks in spite of 0.18 gm. of thyroid daily. A single observation on EsB at 17 weeks, while she was receiving 0.12 gm. of thyroid daily, was 5.0 γ per cent. The remaining determination on RBl, at 19 weeks, while she was receiving 0.12 gm. of thyroid, was 5.8 γ per cent, practically indistinguishable from 6.0 γ per cent. In one patient, BE, SPI of 5.6 γ per cent at 16 weeks, without medication, fell to 3.7 γ per cent at 24 weeks on 0.12 gm. of thyroid. This suggests that she disposed of exogenous thyroid with great facility. Such sporadic low values in the later stages of pregnancy have been noted earlier. In most instances SPI appears to be highly responsive to the administration of thyroid. It is not, however, possible in most of these to distinguish the effects of thyroid from the influence of pregnancy itself. The SPI of ACo, for example, before pregnancy, when she was not receiving thyroid, was 7.2 γ per cent. In her first pregnancy, for no clear reason, she was given 0.18 gm. of thyroid at nine weeks. Without any change of this dose, SPI was successively 7.5, 8.5, and 9.4 γ per cent at 13, 19, and 32 weeks respectively. This patient is remarkable in certain other respects. During the course of the year succeeding her delivery, without thyroid, she had an abortion. In a subsequent pregnancy, which is recorded in the Table, her SPI at six weeks was only 4.7 γ per cent. On 0.18 gm. of thyroid it rose to 5.9 γ per cent after a week and to 8.3 γ per cent at 17 weeks, approximately the same as its concentration at the same stage of her first pregnancy. GGl was given 0.24 gm. of thyroid daily at seven weeks, when her SPI was 5.6 γ per cent. Without any change of dose, the SPI rose

TABLE VI

Patients who received thyroid because of low SPI

Patient	SPI	Weeks*	Thyroid given†		Remarks
			Before	After	
	γ per cent		*gm./day*	*gm./day*	
ACo1	7.2	a 46	0	0	
	7.5	13	0.18	0.18	
	8.5	19	0.18	0.18	
	9.4	32	0.18	0.18	
ACo2	4.7	6	0	0.18	
	5.9	7	0.18	0.18	
	8.3	17	0.18	0.18	Thyroid reduced to 0.06 after 28 weeks
GGl	5.6	7	0	0.24	
	6.4	9	0.24	0.24	
	10.9	12	0.24	0.24	
	8.3	15	0.24	0.24	
	7.0	25	0.24	0.24	
	6.9	32	0.24	0.24	
	5.2	p 16	0		No thyroid after delivery
EH	5.6	a 5	0		
	4.6	7	0	0.18	
	7.6	11	0.18	0.18	
	6.8	15	0.18	0.18	
	7.6	25	0.18	0.18	
B95180	5.7	15	0	0	
	6.6	22	0.18	0.24	
	6.9	36	0.24	0.24	
	4.6	p 38	0		No thyroid after delivery
GM	5.2	4	0	0.12	
	5.9	12	0.12	0.12	
	5.2	17	0.12	0.12	
MR	5.8	6	0	0.12	
	9.7	12	0.12	0.12	
C23634	4.2	a 1	0	0	
	5.2	8	0	0.18	
	7.1	19	0.18	0.18	
EV	5.3	a 12	0	0	
	4.0	6	0	0	
	5.6	7	0	0.24	
	7.5	7	0.24	0.24	3 days later
	10.0	13	0.24	0.18	
EWa	5.3	13	0	0.12	
	6.5	15	0.12	0.18	
	4.7	18	0.12	0.18	On only 0.12 for 1 week
	7.7	29	0.18	0.18	
	5.4	p 8	0		No thyroid after delivery
JW	5.9	10	0	0.18	
MC	3.2	6	0.12	0.30	
	6.9	15	0.30	0.30	
MPe	3.7	6	0	0.18	
EsB	5.0	17	0.12	0.12	
RBl	5.7	5	0	0.12	
	5.8	19	0.12	0.12	
JB	5.8	6	0	0.12	
BE	5.6	16	0	0.09	
	3.7	24	0.09	0.09	
TI	5.6	6	0.12	0.24	
	8.7	16	0.24	0.12	
SJ	4.9	6	0	0.18	
CK	5.2	8	0	0.12	
ML	5.4	13	0	0.18	
	7.3	23	0.18	0.12	
JP	5.8	13	0	0.09	
	8.3	20	0.09	0.09	
KW	4.2	8	0.09	0.09	
GGe	5.5	7	0.12	0.12	
	6.9	13	0.12	0.12	

* a indicates before pregnancy, p indicates after delivery.
† *Before* indicates that the patient was already receiving thyroid. *After* indicates that thyroid was begun after the measurement of SPI.

TABLE VII

Patients who received thyroid and delivered normally, although no SPI lower than 6.0 γ per cent was observed

Patient	SPI	Weeks*	Thyroid given†		Remarks
			Before	After	
	γ per cent		gm./day	gm./day	
JK	6.2	18		0.25	
	6.8	23	0.25	0.25	
HL	7.6	19			
	7.6	26			
	5.5	31	0.12	0.12	
	4.8	32	0.12	0.12	
DM	6.0	30	0.06	0.06	
	7.2	p 30	0.06	0.06	
	6.4	p 100	0.06		
HP	5.1	a 17			
	6.3	7	0	0.18	
	9.9	9	0.18	0.18	
	5.8	20	0.18	0.18	
CP	6.0	11	0	0.12	
	5.9	16	0.24	0.24	0.24 begun after 14 weeks
	6.3	20	0.24	0.24	
HT	6.1	9	0	0.18	
	7.1	12	0.18	0.18	
	6.6	16	0.18	0.18	
C24722	7.3	30	0.18	0.18	Thyroid started at onset of pregnancy
	6.4	37	0.18	0.18	
EKo	6.3	6	0	0.06	0.18 begun after 11 weeks
	9.2	18	0.18	0.18	
MN	4.9	a 124	0.06	0.06	
	6.5	17	0.06	0.12	
	7.8	20	0.12	0.12	
	8.3	27	0.12	0.09	
CR	6.1	10	0.12	0.12	
	5.5	13	0.12	0.24	
RR	6.3	a 22			
	6.2	8	0.12	0.18	
HB	7.3	12	0.06	0.06	
LE	7.3	8	0	0.06	
MPl	6.6	8	0	0.09	
	6.4	19	0.09	0.03	
DS	6.7	13	0	0.06	

* If not otherwise marked, indicates weeks after last menstrual period; a indicates weeks before conception; p indicates weeks after delivery.

† *Before* indicates that the patient was already receiving thyroid. *After* indicates that thyroid was begun after the measurement of SPI.

to a peak of 10.9 γ per cent at 12 weeks and thereafter gradually declined to 6.9 γ per cent at 32 weeks. After delivery, when thyroid had been discontinued, it fell still further, to 5.2 γ per cent. This suggests that, although thyroid may accelerate the initial rise of SPI, thereafter, on a constant dose, SPI follows the course usual in pregnancy. In spite of the fact that doses of from 0.18 to 0.30 gm. of thyroid were given to these subjects over long periods, SPI did not exceed the concentrations ordinarily encountered in pregnancy nor were symptoms or signs of hyperthyroidism encountered. This may denote that the pregnant woman is tolerant of the thyroid hormone or able to dispose of it with unusual facility. It must be recognized, however, that a proportion of normal women have a similar tolerance (10, 11).

If, as other evidence suggests, failure of SPI to rise is incompatible with the continuation of pregnancy, the data of Table VI suggest that administration of thyroid in generous amounts when SPI is low may prevent abortion. This would imply that the increment of SPI in pregnancy is composed of thyroid hormone. To verify this it is necessary, however, to prove more definitely that abortion is a regular consequence of a persistent low SPI. This requires the study of a larger number of untreated subjects in the early weeks of pregnancy.

Fifteen women who delivered normally were given thyroid in spite of the fact that their SPI's were 6.0 γ per cent or higher at the first observations during pregnancy, usually because of histories of previous infertility or abortions. The data from these cases are presented in Table VII. In no instance did the thyroid drive SPI above the normal range for pregnancy. In two, in fact, SPI fell after the administration of thyroid. In HL the low values were observed in the last trimester of pregnancy. In this case thyroid was not given until the end of 27 weeks. In HP, after rising from 6.3 to 9.9 γ per cent between seven and nine weeks on 0.18 gm. of thyroid, SPI dropped to 5.8 γ per cent at the end of 20 weeks. Its subsequent course is unknown.

In both these last series shown in Tables VI

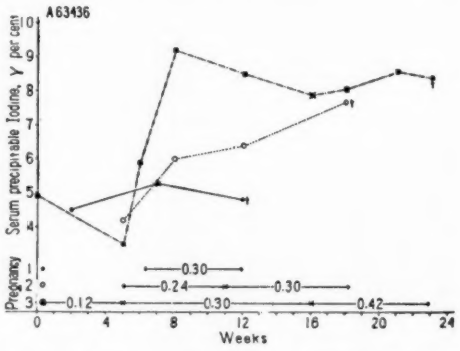

FIG. 2. THE COURSE OF SPI OF A SUBJECT IN THREE PREGNANCIES THAT ENDED IN ABORTION

The daily dose of thyroid, in grams, given during each pregnancy, together with the duration of its administration, is shown by the figures and arrows below the curves. Crosses indicate bleeding or cramps; daggers represent abortion.

and VII, as in previous series, SPI before and after pregnancy, when it was measured, lay within the normal nonpregnant range.

That thyroid may not be successful in preventing abortion, even if it is given in large doses before any signs or symptoms appear, is illustrated by the woman whose record is illustrated in Figure 2. In her first pregnancy, at two weeks and seven weeks SPI's were 4.5 and 5.3 γ per cent, respectively. At this time, because of bleeding, she was given 0.30 gm. of thyroid daily. In spite of this she aborted and curettage was necessary at 12 weeks. Three months later she again became pregnant. After five weeks, because SPI was only 4.2 γ per cent, she was given 0.24 gm. of thyroid. At eight weeks SPI had risen to 6.0 γ per cent. Two weeks later the dose of thyroid was raised to 0.30 gm. Although SPI was 7.7 γ per cent at 18 weeks, she again aborted. Three months later, without thyroid, SPI was 4.9 γ per cent, a normal value. Nevertheless, she was given 0.12 gm. of thyroid daily. Her last menstrual period occurred two days later. At the end of five weks SPI was only 3.5 γ per cent, although the dose of thyroid had been increased to 0.24 gm. three days earlier. It was immediately raised to 0.30 gm. In another two weeks SPI was 9.2 γ per cent. In spite of the fact that it did not fall below 8 γ per cent, uterine contractions began at 16 weeks. These diminished after the dose of thyroid was increased to 0.42 gm., but she aborted after 23 weeks. In this case the rise of SPI in every pregnancy was delayed. In fact, in every instance it was lower than the only value obtained in the nonpregnant state. This was within normal limits, an indication that the subject had no thyroid deficiency. Nevertheless, large doses were required to raise SPI to characteristic pregnancy values. Even when thyroid was begun before pregnancy and relatively early elevation of SPI was achieved, abortion was not prevented. As the intensity of thyroid therapy was increased and as it was initiated at an earlier date, there was a suggestion that pregnancy was prolonged. In this case there appears to be some inherent obstacle to the conduct of normal pregnancy that is associated with low SPI. This can be ameliorated, but not abolished, by administration of enough thyroid at an early date to raise SPI to concentrations appropriate for pregnancy.

TABLE VIII

Premature deliveries

Patient	SPI	Weeks*	Thyroid given†		Remarks
			Before	After	
	γ *per cent*		*gm./day*	*gm./day*	
KA	8.8	25	0.15	0	Spotting for 6 days; delivery 2 days after SPI.
19373	6.5	17	0	0	Slight bleeding 4 weeks earlier; delivery at 25 weeks.
VH	5.1	6	0.06	0.21	
	8.2	12	0.21	0.21	Delivery at 36 weeks
B59643	7.7	22	0	0	Delivery at 35 weeks
MRo	9.9	16	0	0	Delivery at 36 weeks

* Weeks after last menstrual period.

† *Before* indicates that the patient was already receiving thyroid. *After* indicates that thyroid was begun after the measurement of SPI.

Similar conditions may obtain in those subjects reported above who aborted in spite of normal SPI.

Premature deliveries. Attention has been called frequently to occasional low values occurring late in pregnancy. It has been impossible to attach any significance to these. Furthermore, to date no relation has been discovered between "premature" deliveries in the latter months of pregnancy and SPI. The data on this point are, however, necessarily scanty. Five patients who received the diagnosis of premature delivery are presented in Table VIII. The first two do not properly belong in this category because both delivered at 25 weeks. The first, KA, just before delivery, on 0.15 gm. of thyroid daily had an SPI of 8.8 γ per cent. The second, 19373, at 13 weeks bled slightly; but at 17 weeks had not aborted and had an SPI of 6.5 γ per cent. She went into labor, however, and delivered at 25 weeks. The remaining three patients delivered at 35 or 36 weeks. One of them, VH, was given 0.06 gm. of thyroid before pregnancy because her SPI was only 3.8 γ per cent, a low normal figure. At six weeks, because SPI had risen only to 5.1 γ per cent, the dose of thyroid was increased to 0.21 gm. daily. The SPI at 12 weeks had risen to 8.2 γ per cent. Although the thyroid was continued, she had a premature delivery after 36 weeks. The SPI's of the remaining two appear to have risen to proper pregnancy concentrations. Their pregnancies were uneventful until their deliveries at 35 and 36 weeks respectively. Neither received thyroid. It is impossible in any

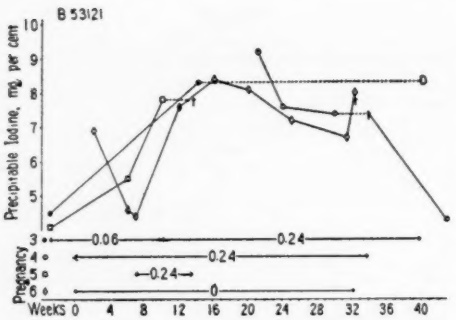

FIG. 3. THE COURSE OF SPI DURING FOUR PREGNANCIES OF A SUBJECT WITH A TENDENCY TO PREMATURE DELIVERY

The general construction of the chart is similar to that of Figure 2. D represents normal delivery.

of these cases to be certain that SPI did not fall later in the course of pregnancy.

One patient, whose record is illustrated in Figure 3, merits especial consideration. At the age of 22, because of a basal metabolism of − 23, she was given 0.0125 gm. of thyroid daily. In 1939, when her basal metabolism was found to be − 12, she was given 0.06 gm. of thyroid daily. On neither occasion did she have symptoms of hypothyroidism. In November, 1942, her right tube was removed for a tubal pregnancy. In February, 1946, she had an abortion at three months. July 22, 1946, while on 0.06 gm. of thyroid daily, her SPI was 4.5 γ per cent. October 24 she became pregnant. Shortly after this the dose of thyroid was increased to 0.24 gm. daily. She had an uncomplicated pregnancy, except for slight bleeding in the first months at the times of her menstrual periods, and was delivered on July 23, 1947, at term, of a normal child. On November 10 of that year she again became pregnant. Thyroid 0.24 gm./day was begun on December 27. Again she bled slightly in the first months at the time of her normal menstrual periods; but otherwise her pregnancy proceeded normally until July 4, 1948, when she went into labor and was delivered of a premature child who died two days later. Thyroid medication was discontinued after delivery. SPI's, 38 and 93 days after delivery, were 4.3 and 4.1 γ per cent, respectively. February 23, 1949, she again became pregnant. After six weeks her SPI was 5.5 γ per cent. She was again given 0.24 gm. of thyroid daily. Four weeks later her SPI was 7.8 γ per cent. Nevertheless she had a spontaneous abortion at the end of 13 weeks. On July 27 she once more became pregnant. On this occasion no thyroid was given. SPI was 6.9 γ per cent only two weeks after her last menstrual period. Although it fell to 4.6 and 4.4 γ per cent at six and seven weeks, it rose after this spontaneously and remained well above 6.0 γ per cent until the end of 32 weeks when she bled profusely. Despite administration of 2 mg. of thyroxine intravenously, she was delivered of a dead fetus within 24 hours, with evidence of premature separation of the placenta. From the values of SPI when she was neither pregnant nor receiving thyroid, as well as the absence of symptoms and signs, the patient was evidently not hypothyroid. There is no evidence that administration of thyroid altered the course of her pregnancies. The fact that one pregnancy went to term while she was receiving thyroid seems to be purely adventitious. Without thyroid, the last, her sixth pregnancy, proceeded quite as long as did the fourth in which she received thyroid.

DISCUSSION

That SPI rises during pregnancy appears to have been established. This rise is attended by no clinical evidence of excessive activity of the thyroid gland. It occurs long before the increase of basal metabolism that accompanies pregnancy. The latter also presumably denotes no increased activity of the thyroid, but seems rather to be related to the need for extra oxygen created by the enlargement of the uterus and the growth of the fetus.

The almost complete absence of SPI's below 6.0 γ per cent after 16 weeks suggests that concentrations above this are, in normal persons, necessary for the successful completion of pregnancy. At the left of Figure 1 the limits of variation of SPI in various conditions are defined. It is evident that in a group of nonpregnant women of this size the majority of points would lie between 4 and 6 γ per cent, an area that is almost empty, and that there would be only an occasional spot above 7 γ per cent, which is the most thickly populated area of the figure. In the period before 12 or 16 weeks there are more points in the normal range. If all pregnant women were in-

cluded, not only those who completed pregnancy without medication, the area between 4 and 6 γ per cent in the first weeks of pregnancy would be fuller. From the paucity of points below 6 γ per cent after 16 weeks, the fact that the SPI of non-pregnant women most frequently lies in this area, and the greater frequency of such points in the earlier weeks of pregnancy, it may be inferred that before the end of this period SPI regularly attains a concentration of 6 γ per cent or more and that when it fails to rise to this level abortion occurs.

In the patients who aborted and those who received thyroid substances, as in the normal pregnancies, there is the same tendency for SPI in pregnancy to lie above 6.0 γ per cent after 16 weeks. This is best illustrated in Figure 4 which includes all observations on patients who were delivered at term. A cross indicates that the subject was receiving thyroid, a dot that she was taking no medication. The paucity of points in the area below 6.0 γ per cent after 16 weeks is again evident. Of the total number of observations only ten are found in this area. Of these, five are greater than 5.0 γ per cent.

There is, however, in contrast to the series of normal pregnancies reported in the preceding paper, a larger aggregation of values below 6 γ per cent in the early weeks of pregnancy. This would be still greater if the observations in Table III from the subjects who aborted were included. The incidence of low points also seems to diminish as pregnancy advances to 16 weeks. SPI, therefore, in a considerable number of women, remains low in the early weeks of pregnancy. Some of the later points below 6 γ per cent represent transient fluctuations. However, the concentration 6.0 was selected somewhat arbitrarily because the great majority of observations lay above this concentration. It would be surprising indeed if there were not some overlap in the zone of differentiation. The two extremely low figures represent single observations on two patients. What the SPI may have been at other times in the course of pregnancy is unknown. On the whole the data support the earlier hypothesis that if pregnancy is to be completed, SPI is not consistently below about 6.0 γ per cent after pregnancy has proceeded approximately 16 weeks. This would mean that SPI regularly achieves this level in the course of the first 16 weeks in successful pregnancies. This suggests, but does not necessarily imply, that failure of SPI to rise in this period conduces to abortion. Definitive proof that this is the case must depend upon the intensive study of a large group of subjects during early pregnancy without medication.

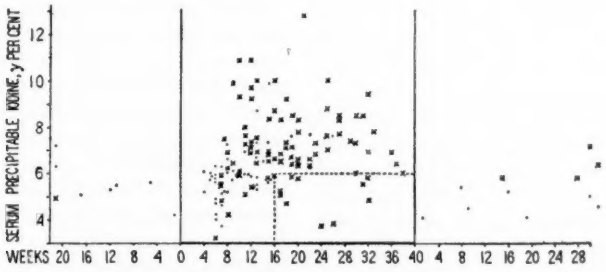

FIG. 4. THE SPI OF ALL SUBJECTS WHO RECEIVED THYROID AND SUCCESSFULLY COMPLETED PREGNANCY

Dots indicate that the subject had not received thyroid, crosses that she had received thyroid before the observation. The period of pregnancy is defined by the vertical lines. The area to the left represents time before, that to the right time after, pregnancy.

In the small series thus far collected, early miscarriage appears to be more common in those with low SPI. An SPI above 6.0 γ per cent, however, obviously does not preclude abortion. If failure of SPI to rise does predetermine abortion, the question arises whether there is a critical time and a critical concentration. Sometimes

SPI exceeds 6.0 γ per cent as early as the diagnosis of pregnancy can be established. In other cases it remains below this concentration until the period of 16 weeks is almost completed. The majority of miscarriages occur earlier than this. If low SPI is correlated with the cause, rather than the incidence, of miscarriage, why do some subjects tolerate low values longer than others do? Are those who do miscarry inherently incapable of raising their SPI's or would their SPI's eventually rise if they escaped miscarriage?

Failure of SPI to rise in those that miscarry may be an indication that there is a defect in the ovum or its implantation of such a kind that the stimulus that causes SPI to rise is lacking. In those in which SPI rises late, but abortion is avoided, this stimulus may be only partly lacking, or the defect may lie not in a deficient stimulus, but in a deficient or delayed reaction of the mechanism responsible for the rise of SPI, presumably the secretory activity of the thyroid gland or the utilization of the thyroid hormone, or both. In this case SPI would be little more than a symbol. Miscarriages would be associated with low SPI, but the latter would not necessarily determine a miscarriage. Such a hypothesis seems to be consistent with most of the facts presented. It gains some support from the cases in which the patient who miscarried in one pregnancy with low SPI had a subsequent successful pregnancy when SPI rose early (cases BH and MG, Tables III and IV). It might partly explain the consistent failure to complete pregnancies displayed by A63436 (Figure 2). It would not, however, account so well for the relative absence of low values after 16 weeks or the apparent prolongation of pregnancy by thyroid in A63436.

This hypothesis would relegate the elevation of SPI and the thyroid hormone to a derivative or adventitious position. Alternatively, completion of pregnancy may depend on the proper reactivity of the mechanism which causes SPI to rise, presumably increased secretory activity of the thyroid gland, with diminished or increased utilization of thyroid hormone. If the first hypothesis is correct, the common practice of administering thyroid to prevent abortion, which originated on a purely empirical basis, would be of no value. These two hypotheses are not, of course, mutually exclusive. It may be that both stimulus and reactivity of the thyroid gland must be adequate. The present study is intended in part to throw some light on these questions.

It is clear from the data of Table III that the administration of thyroid, or even thyroxine, in massive doses, will not regularly prevent abortion after symptoms have begun. In most instances, however, there can be no assurance that abortion was not already an accomplished fact before the thyroid or thyroxine was given. The generally higher values of SPI in the successful pregnancies of Table IV, as contrasted with those in the abortive pregnancies of the same individuals in Table III, suggest that a high SPI is advantageous and that administration of thyroid tends to raise SPI. The patients in Table V might be cited as more direct evidence of the beneficial effect of thyroid. In this group completion of abortion may have been prevented by timely administration of thyroid. In five of the ten pregnancies, medication was followed by a distinct rise of SPI. In three of the five, SPI at the onset of cramps or bleeding was lower than 6 γ per cent. The total number of cases is, however, small and the number of controls who, under similar circumstances, did not receive thyroid is smaller. In Tables VI and VII is a goodly array of women who, having received thyroid, completed pregnancy successfully without symptoms of miscarriage at any time. The greater proportion of these (those in Table VI) were given thyroid because SPI was low. In most of those in which further observations were made, SPI rose in response to the medication, but repeated observations were all too few and again adequate controls are lacking. The data on the whole suggest, but certainly do not prove, that administration of thyroid prevents abortion.

Attention has been called to the incidence of abortions with SPI above 6 γ per cent. If low SPI does promote abortion, these cases can not be summarily dismissed as belonging to a different category. In normal pregnancy some women carry higher SPI than others. This may be a necessary provision. In only one of the patients of this series, in addition to A63436 (Figure 2), did abortion or threatened abortion occur when SPI was 7.5 γ per cent or higher. It may be more than a coincidence that the points below 6 γ per cent after 16 weeks in Figure 4 are all

derived from patients who were receiving thyroid. If the increment of SPI in pregnancy is composed of thyroid hormone, its significance still remains to be elucidated. It could mean diminished utilization of hormone, though this seems highly unlikely. It is generally held, from morphological studies, that secretory activity of the thyroid is increased and, as a corollary, that this is a response to an increased demand for the hormone to meet increased utilization. An increased supply would then be the prime necessity. With the administration of exogenous thyroid the tissues might be able to derive the necessary hormone from the circulation by more efficient extraction with a lower concentration in the serum.

Much of this discussion and the data on which it is based tend to pose rather than to answer questions as to the clinical value and significance of SPI in pregnancy. The data may, however, throw some light on the nature of the increment in pregnancy. Values of SPI as high as those encountered in pregnancy are regularly associated with symptoms of hyperthyroidism in nonpregnant women. This seems to denote either that the pregnant woman tolerates a greater concentration of hormone in her serum or that the increment is not composed of thyroid hormone. In many of the cases in this series, SPI was rapidly raised by administration of thyroid or thyroxine to levels characteristic of hyperthyroidism without appearance of symptoms. Presumably these increments of iodine were hormonal. Pregnancy does, therefore, appear to confer a tolerance for circulating thyroid hormone. In a few instances in which SPI was suddenly raised to unusually high concentrations it tended to decline to more moderate concentrations as pregnancy advanced. Whether the endogenous supply was reduced, utilization accelerated, or removal by other channels increased, it is impossible as yet to say. It is noteworthy that on the whole, regardless of the dose of thyroid, the concentration of SPI, with the exception of the temporary shoots mentioned above, tended to establish itself in the range characteristic of pregnancy.

This tolerance appears to differ somewhat from that displayed by nonpregnant women, in which the concentration of SPI does not rise, or, after a preliminary rise, recedes to the normal range (10). In most of the present series in which SPI was measured postpartum, thyroid was discontinued after delivery. In the two cases in which it was continued, it was found to be in the normal nonpregnant range. In one it fell after delivery. This strengthens the impression that SPI is sustained by pregnancy. Whether the increment of SPI is, like the greater proportion of the normal SPI, composed of thyroid hormone cannot be decided from these studies. It does behave like the thyroid hormone in attaching itself to the serum proteins. Furthermore, it is extracted by butanol (12, 13).

CONCLUSIONS

The serum precipitable iodine has been determined on one or more occasions during pregnancy in 46 women without complications or signs of overactivity or underactivity of the thyroid gland who delivered at term. In only three instances was SPI observed below 6.0 γ per cent after 16 weeks of pregnancy. In nine patients with pregnancies complicated by conditions other than abnormal activity of the thyroid, SPI with one exception lay at the same high levels. This is also true of 14 patients still undelivered but with apparently normal pregnancies, well advanced. In those patients studied before and after pregnancy, SPI is found to rise early in pregnancy and to fall shortly after delivery.

The precipitable iodine of serum (SPI) has been measured in a series of euthyroid pregnant women who aborted or who received thyroid substance to prevent abortion. There is some evidence that if SPI does not rise above about 6 γ per cent during the first 16 weeks, pregnancy is unlikely to proceed to term and that abortion is more common in those women whose SPI's do not rise relatively early in pregnancy. Administration of generous amounts of thyroid substance accelerates the rise of SPI. The cases reported suggest that, if thyroid is given early enough, it may diminish the tendency to abortion. A larger body of controls is required to establish these points.

If thyroid is given to the pregnant woman, the curve of SPI tends to follow a course similar to that of the untreated pregnant woman. The implications of this fact with respect to the nature

and significance of the increment of SPI in pregnancy are discussed.

ACKNOWLEDGMENTS

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THE PRECIPITABLE IODINE OF SERUM IN PREGNANCY COMPLICATED BY DISORDERS OF THE THYROID [1]

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In a series of papers (1–3) it has been shown that the precipitable iodine of the serum (SPI) rises in early pregnancy and remains elevated until delivery, thereafter returning to its usual concentration after a variable, but usually short, interval. Although SPI may rise to concentrations usually associated in nonpregnant women with hyperthyroidism, in pregnancy these rises are accompanied by no marks of excessive thyroid activity. In a large series of subjects without disorders of the thyroid, SPI was regularly below 7.8 γ per cent, in hyperthyroid subjects it was regularly above 7.2 γ per cent. Between these two points there was slight overlapping (4). In normal pregnancy, however, the majority of values lie above 7.2 γ per cent, with some as high as 10.0 γ per cent (3). The evidence indicates that failure of SPI to attain a concentration of about 6.0 γ per cent or higher within the first 16 weeks of pregnancy predisposes to miscarriage, and suggests that administration of active thyroid substances to women with low SPI early in pregnancy may avert miscarriage (3).

The significance of the elevated SPI is quite obscure. This increment is presumably composed of thyroid hormone. If it is hormone it still remains to discover whether the supply of hormone from the thyroid gland is increased, whether its utilization by the tissues is increased or diminished, or whether for some reason its combination with serum protein is unusually indissoluble. Other explanations of the phenomena can be conceived, but these serve to indicate the nature of the problem. Some indirect evidence may be secured by analysis of the behavior during pregnancy of patients with excessive or deficient thyroid activity. The present paper, therefore, deals with the course of SPI in pregnancy of 16 patients with disorders of function of the thyroid gland.

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MATERIAL AND METHODS

The 16 patients were all under the care of the Metabolism Clinic and staff. The majority were delivered on the Obstetrical Service of Yale University School of Medicine in the New Haven Hospital. A small number were delivered elsewhere. The procedures and methods have been already described (3).

Of the 16 patients nine were definitely hyperthyroid. Of these, one had two, and two had three pregnancies. One patient was definitely, a second probably, and three others questionably, hypothyroid. One patient when first seen in pregnancy had indubitable hyperthyroidism, but after three pregnancies became hypothyroid. The last of the 16 had questionable hyperthyroidism. In all cases the treatment was altogether medical, consisting of the administration of dried thyroid, Lugol's solution, and either thiourea or propylthiouracil. With few exceptions, which are noted in the protocols, patients received 14 or 15 drops of Lugol's solution daily while they were taking thio drugs.

PROTOCOLS OF CASES

Only features relevant to the subject of this paper are included in the following protocols.

B82863, born in 1917, had had a thyroidectomy for toxic goiter in 1939 and two operations for recurrences in 1946; one uncomplicated pregnancy in 1943, miscarriages in 1944 and 1946. When first seen, June 26, 1946, she had frank signs of hyperthyroidism with a diffuse goiter, slight exophthalmos, blood pressure 160/90, basal metabolism +26 per cent, and SPI 12.5 γ per cent. The record of SPI and antithyroid medication, beginning at this point, is illustrated in Figure 1. The course for 66 weeks preceding pregnancy is represented in broken lines to show the fluctuant character of the disease and SPI. During this period she was treated intermittently with Lugol's solution alone. When SPI was below 8.0 γ per cent she was free from signs and symptoms; when it rose above 8.0 γ per cent, hyperthyroidism manifested itself. The SPI of 8.8 γ per cent just before preg-

nancy 4 was accompanied by symptoms and apparently marked an exacerbation of her disease, since thio drugs were required thereafter. Throughout the pregnancy and the early postpartum period she seemed to be in a euthyroid state; but 16 weeks after delivery, with SPI 10.3 γ per cent, there were signs of overactivity of the thyroid. Nevertheless, medication was stopped. She was evidently in a relapse 11 weeks before pregnancy 5 with SPI 18.4 γ per cent. This responded rapidly to propylthiouracil, the dose of which had to be reduced in the course of the pregnancy. Symptoms of hyperthyroidism did not recur until after 26 weeks and following interruption of the drug for two weeks SPI reached 13.2 γ per cent. Symptoms ceased again shortly after the SPI of 10.3 γ per cent at the end of 31 weeks.

The hyperthyroidism in this case evidently progressed in severity during the period before pregnancy 4. The outburst of hyperthyroidism before pregnancy 5 can be attributed to inadvised discontinuation of therapy. The doses of thio drugs used in the two pregnancies are not so different as the figures would indicate because of the greater effectiveness of a given weight of thiourea. The initial dose was probably greater in pregnancy 5 while the later dose was greater in pregnancy 4 until the last period of 5 when the dose of propylthiouracil was increased to 100 mg. This may account for the differences in the two pregnancies. On the whole, however, the general similarity of the course of SPI is more striking. It fell rapidly in the early part of both pregnancies, despite diminishing doses of thio drugs, until it reached values lower than those usually encountered in pregnancy, rising again in the latter half of pregnancy. The sequences are complicated by the fact that the patient was in a hyperthyroid state just before both pregnancies. Initial treatment was obviously overvigorous at first and, in pregnancy 5, too light later. The complete withdrawal of propylthiouracil in this pregnancy was an error of clinical judgment. The rises of SPI as pregnancy advanced may have been due in part to the influence of pregnancy itself. In both pregnancies the patient remained free from symptoms when SPI lay between 8 and 10 γ per cent, the range for normal pregnancy, although symptoms of hyperthyroidism had appeared with SPI in the region of 8 γ per cent when she was not pregnant. This tolerance persisted for an interval after delivery in both instances, while SPI remained elevated. Subsequently, after pregnancy 4, hyperthyroidism appeared, with a further rise of SPI.

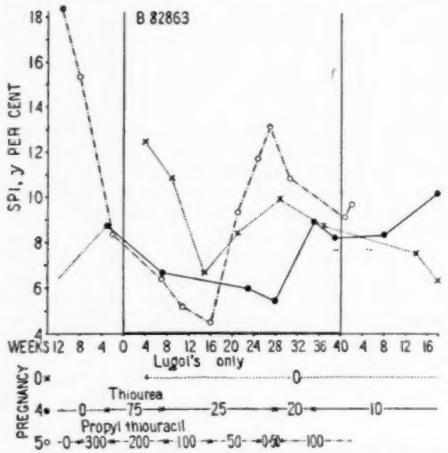

FIG. 1. THE COURSE OF SPI OF A HYPERTHYROID PATIENT BEFORE AND DURING TWO PREGNANCIES

The period of pregnancy is defined by the two vertical lines. It is arbitrarily set at 40 weeks after the last menstrual period, 0 weeks. Observations are also dated from this period. The interval between the last observation in pregnancy and delivery is not, therefore, precise, since delivery did not always occur after exactly 40 weeks. Periods before and after pregnancy are indicated to the left and right of the pregnant area, respectively. Therapy is indicated by the figures below the chart, the lines and arrowheads indicating the duration of each dose.

In this particular case the broken line with solid dots does not represent a pregnancy, but a continuous period of 72 weeks preceding her fourth pregnancy during which the patient received only Lugol's solution (see protocol).

B50050, born in 1922, had normal deliveries in 1941 and 1942, miscarriages in 1941, 1942 and about July of 1945. Symptoms of hyperthyroidism began early in 1945. When first seen in March, 1946, she had diffuse enlargement of the thyroid, slight exophthalmos, frank hyperthyroidism, basal metabolism +68 per cent and SPI 24.2 γ per cent. The course of SPI and therapy, beginning with this point, which just preceded pregnancy, is illustrated in Figure 2. After six weeks, with SPI down to 9.3 γ per cent, symptoms and signs of hyperthyroidism had subsided. In neither pregnancy did symptoms or signs recur until the sharp peaks of SPI at 10 and 11 weeks postpartum, respectively.

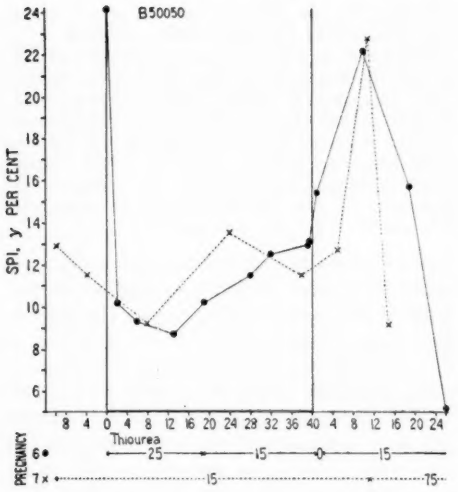

FIG. 2. THE COURSE OF SPI OF A HYPERTHYROID PATIENT IN TWO SUCCESSIVE PREGNANCIES

The general significance of the symbols is described in the legend to Figure 1. Only a part of the interval between pregnancies is illustrated.

The similarity of the course of SPI in these two pregnancies seems too close to be ascribed to coincidence. The slight early decline and the late rise resemble the reactions of the preceding case (B82863), but the picture is not distorted by changes of therapy or the severity of the thyroid disorder, which appears to have remained relatively stable. The impression is derived that in pregnancy hyperthyroidism can be more easily controlled. In this sense hyperthyroidism is not synonymous with SPI which appears to follow a course similar to that characteristic of pregnancy in euthyroid subjects, but perhaps at a higher level. The outbursts of hyperthyroidism after each delivery suggest a discharge of activity after the release of the inhibitory effect of pregnancy. The figures in the later part of pregnancy are so high as to indicate that this condition conferred a degree of tolerance for thyroid hormone (or SPI) greater than the similar tolerance of euthyroid pregnant subjects. In this case, however, this can not be definitely substantiated. Just before pregnancy 7, while the patient appeared to be in a euthyroid state, values of 12.9 and 11.5 γ per cent were observed.

B35786, born in 1922, had a normal pregnancy in 1943. In 1940 and in 1944 she was given small doses of thyroid because of increasing weight and lethargy. Symptoms of hyperthyroidism appeared early in February, 1946. She was seen March 7 of that year, four weeks pregnant, with moderate enlargement of the thyroid, typical manifestations of hyperthyroidism, basal metabolism, + 56 per cent and SPI 23.1 γ per cent. Her course, beginning with this point, is illustrated in Figure 3. There was a temporary sharp fall of SPI, but symptoms were not allayed and basal metabolism remained elevated until about the 24th week. She was again distinctly hyperthyroid 11 weeks after delivery when SPI had shot up to 23.8 γ per cent and continued to have symptoms, except during a short remission when SPI fell to 6.2, until pregnancy 3. In the first part of this pregnancy, which terminated in a spontaneous miscarriage after 23 weeks, hyperthyroidism was not allayed and SPI rose, probably because the patient was unable by reason of nausea to take her medication. Three days after the miscarriage (the interval in the figure is not real, but represents only transition from pregnancy to the postpartum state) basal metabolism was + 3 per cent and SPI had fallen to 11.1 γ per cent. Thirty-five weeks after the miscarriage she was again thyrotoxic and came into pregnancy 4 in this state. She became euthyroid, however, shortly after pregnancy began and remained in this state as long as she was followed.

In this case again, after the initial hyperthyroidism was controlled, SPI declined steadily in both pregnancy 2 and pregnancy 4, remaining, however, always above nonpregnant euthyroid con-

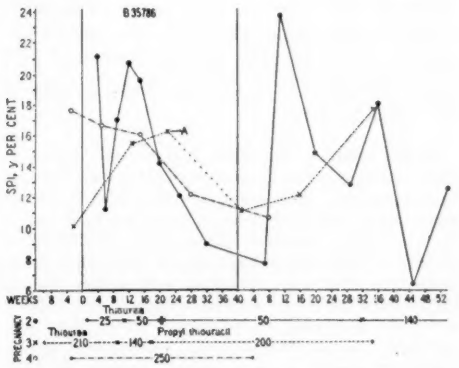

FIG. 3. THE COURSE OF SPI OF A HYPERTHYROID PATIENT IN THREE SUCCESSIVE PREGNANCIES

The general significance of the symbols is described in the legend to Figure 1. Pregnancy 3 ended in abortion at the point *A*. The interval between this and the vertical line marking the end of pregnancy does not indicate actual elapsed time. The next observation was made one week after the abortion.

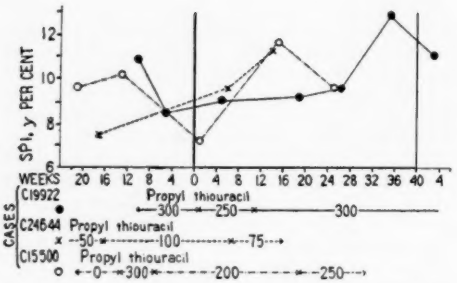

FIG. 4. THE COURSE OF SPI OF THREE HYPERTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1.

centrations, suggesting that the requirement for thio drug diminished. Pregnancy 3 can not be evaluated. The course of SPI might suggest that the hyperthyroidism was exacerbated, the rise of SPI can probably be attributed to her failure to take her medication. After pregnancy 2 she had an outburst of hyperthyroidism associated with a sharp rise of SPI quite similar in degree and time to those of the preceding case. This only partly subsided spontaneously. Although no sharp spike was noted after pregnancy 3, SPI rose after pregnancy. At 36 and 35 weeks, respectively, the values for SPI after pregnancies 2 and 3 were almost identical. The patient appeared to tolerate while pregnant values of SPI higher than those tolerated even during pregnancy by normal persons.

C19922, born in 1913, when first seen on January 4, 1949, had four children, the oldest 11. Symptoms of hyperthyroidism, which began four months earlier, were characteristic, including a slight goiter and mild exophthalmos, basal metabolism +34 per cent and SPI 10.9 γ per cent. Her course beginning at this point is illustrated in Figure 4. Symptoms were rapidly allayed, but recurred when the dose of thiouracil was reduced during the early part of pregnancy.

C24644, born in 1914, developed symptoms of hyperthyroidism shortly after a normal delivery in 1946. When first seen, April 16, 1948, after she had taken 150 mg. of propylthiouracil daily for three weeks, she was still definitely hyperthyroid, with a diffusely enlarged thyroid and SPI 7.1 γ per cent. On Lugol's solution and 50 to 125 mg. of propylthiouracil daily, symptoms and signs were kept in abeyance and SPI within normal limits until the onset of her pregnancy, January 17, 1950. The record in Figure 4 begins 17 weeks before this. Although, after the onset of pregnancy, without change of medication, SPI rose, she remained euthyroid.

C15500, born in 1920, had a normal pregnancy in 1946. In 1942 she developed hyperthyroidism, which recurred after a thyroidectomy in 1944. When first seen, August 30, 1948, she had mild symptoms of hyperthyroidism and SPI 9.6 γ per cent. The course beginning with this point is illustrated in Figure 4. Symptoms subsided rapidly after propylthiouracil was begun. Thereafter, with the exception of a short interval at about 16 weeks when SPI was 11.7 γ per cent, she seemed euthyroid.

SPI in the three cases illustrated in Figure 4 remained at more moderate concentrations than it did in the three preceding cases. The observations were, however, limited. In all, insofar as they were studied, SPI tended to rise as pregnancy advanced. With these rises C19922 and C15500 developed or retained some symptoms and signs suggestive of continued overactivity of the thyroid. These are the only patients in the series who had such manifestations while SPI was below 10 γ per cent. In contrast, SPI of C24644 rose during pregnancy, without evidences of hyperthyroidism, to concentrations far above those that before pregnancy had been associated with symptoms.

C38870, born in 1919, had an uncomplicated pregnancy in 1942. At that time she was given 0.03 gm. of thyroid daily. In November, 1944, she developed symptoms of hyperthyroidism, accompanied by urticaria, angioneurotic edema and bronchial asthma. When seen in this condition September 9, 1945, her basal metabolism was +36 per cent, SPI 10.4 γ per cent. The record of her course in Figure 5 begins eight weeks before the onset of pregnancy, which began June 15, 1949. In spite of the steady rise of SPI during pregnancy, she had no evidences of hyperthyroidism until eight weeks after delivery when SPI was 16.1 γ per cent.

B87633, born in 1909, had normal pregnancies in 1940 and 1943. After a thyroidectomy in 1940, hyperthyroidism recurred in 1945. When first seen, May 29, 1946, 23 weeks after the beginning of pregnancy, she had a diffuse goiter, basal metabolism +24 per cent and SPI 13.3 γ per cent. Her course beginning with this point is illustrated in Figure 5. She had a remission of symptoms shortly after medication began and just after delivery, corresponding to the low SPI's, but had a slight recurrence of thyrotoxicosis just before delivery and a more obvious one eight weeks after delivery.

ST, born in 1918, developed hyperthyroidism in 1944. When first seen, May 8, 1947, taking probably 150 mg. of propylthiouracil and 15 drops of Lugol's solution daily, her thyroid was moderately enlarged, but she had no signs of hyperthyroidism. SPI proved to be 1.9 γ per cent. The course, beginning with this point, nine weeks before pregnancy, is illustrated in Figure 5.

With reduction of the dose of propylthiouracil SPI rose quite slowly at first, but between 11 and 16 weeks jumped from 4.9 to 9.2 γ per cent. Just before delivery, the dose of propylthiouracil was increased because of transitory equivocal symptoms of hyperthyroidism: palpitation and sweating, but no tachycardia, basal metabolism + 5 per cent and SPI 8.3 γ per cent. With the SPI 20.6 γ per cent 12 weeks after delivery she was frankly hyperthyroid with pulse rate 102 and basal metabolism + 55 per cent. The infant at birth had a large goiter. This will be discussed in another communication.

The three cases in Figure 5 suggest again that the requirement for thio drugs, estimated by clinical criteria, diminishes, although SPI may rise, as pregnancy advances. They also suggest that SPI may rise higher in pregnancy and for a short interval after pregnancy than in the nonpregnant state, without evidence of hyperthyroidism. They also suggest that the hyperthyroid patient can tolerate higher values than the euthyroid subject can during pregnancy. In case C38870 reduction of thiourea from 75 to 25 mg. may have been unwarranted. Nevertheless, it resulted in no precipitate rise of SPI, but a gradual, symptomless ascent from 8.4 to 12.1 γ per cent. Case B87633, on 15 mg. of thiourea daily, was not unquestionably euthyroid when SPI fluctuated between 13.3 and 10.2 γ per cent; but, after the first observation, signs of hyperactivity were minimal. After delivery all three patients developed hyperthyroidism, C38870 and B87633 without change of therapy, ST despite an increased dose of thiourea. In B87633 these symptoms were not associated with a greatly elevated SPI, but in the other two patients they were. ST exhibited a sharp peak similar to those observed in B50050 (Figure 2) and B35786 (Figure 3) at approximately the same interval after delivery. ST, when first seen, was evidently approaching myxedema. Reduction of the dose of propylthiouracil to 100 mg. raised SPI only to 4.9 γ per cent in 15 weeks. At the end of that time, however, it rose abruptly without change of therapy, behaving like that of a normal pregnant woman.

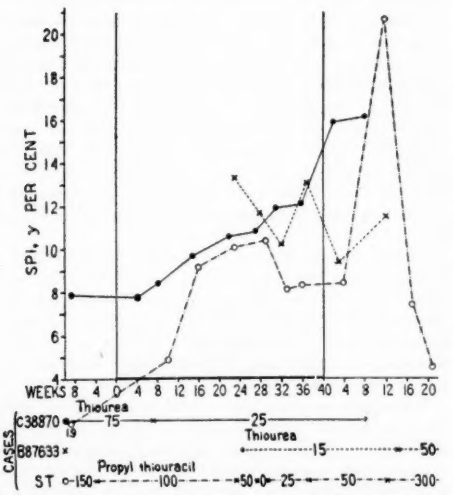

FIG. 5. THE COURSE OF SPI OF THREE HYPERTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1.

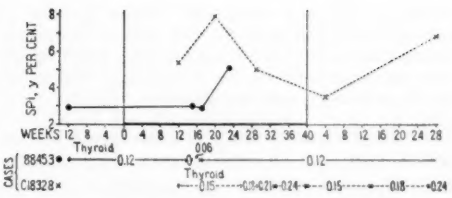

FIG. 6. THE COURSE OF SPI OF TWO HYPOTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1.

88453, born in 1919, had a pregnancy in 1938. In 1939 she noted increasing weight, puffiness of the eyelids and legs, and increasing fatigue. When first seen on September 16, 1942, she presented classical signs of myxedema. SPI was 0.4 γ per cent, basal metabolism − 33 per cent and serum cholesterol 249 mg. per cent. On 0.12 gm. of thyroid daily her condition improved rapidly, SPI varying from 3.0 to 5.4 γ per cent, basal metabolism from − 14 to + 7 per cent, in the next 15 months. She became pregnant January 1, 1944. SPI 11 weeks before this was 2.9 γ per cent. Her course, beginning with this point, is illustrated in Figure 6. Subsequent SPI's in 1949, while she was still taking 0.12 gm. of thyroid, were 3.0 and 3.4 γ per cent. Clinically she remained euthyroid throughout pregnancy on the same dose of thyroid that she took when not pregnant.

C18328, born in 1922, in 1944 noted increasing weight, somnolence, lassitude, dry nails and brittle hair. In 1945, 0.06 gm. of thyroid daily, given because her metabolism was − 26 per cent, restored her to a normal state. When she stopped the thyroid that summer symptoms recurred, basal metabolism falling to − 28 per cent. She was given 0.12 gm. of thyroid, which was increased

in March, 1946, to 0.15 gm. daily. When first seen on November 15, 1948, after the 12th week of pregnancy, she appeared normal in all respects, with SPI 5.4 γ per cent and basal metabolism −11 per cent. The record in Figure 6 begins with this point.

Of the two cases illustrated in Figure 6, one, 88453, had frank myxedema; the second probably had hypothyroidism. Unfortunately, the observations on both, in and about pregnancy, are scanty. Case 88453 had extremely low SPI's at 15 and 17 weeks, 3.0 and 3.4 γ per cent, respectively. This would seem to indicate that such values are not incompatible with completion of a normal pregnancy. She had, however, omitted thyroid for nine days before the first of these and had taken an inadequate dose in the interval of three weeks before the second. The two low values may, therefore, represent a transitory drop. With resumption of a proper maintenance dose, SPI at the next observation was 5.1 γ per cent and may have risen farther as pregnancy advanced. Case C18328, when first seen in pregnancy, had an SPI of 5.4 γ per cent that rose, at the end of eight weeks, without change of therapy, to 7.9 γ per cent. In the later part of pregnancy it had fallen to 5.0 γ per cent, although the dose of thyroid had been increased. Unfortunately, no further observations were made until after delivery when, on the original dose of thyroid, SPI was 3.5 γ per cent. This patient was never seen when she was not taking thyroid. The story of her initial symptoms and her reactions to administration and withdrawal of thyroid seem characteristic of hypothyroidism. Nevertheless, SPI was never subnormal. Furthermore, it rose spontaneously under the stimulus of pregnancy, as it does in normal persons, without change of therapy, and fell after delivery. Finally, she was able after delivery to tolerate 0.24 gm. of thyroid without clinical evidence of hyperthyroidism, which is unusual for a hypothyroid subject. The effect of this dose on SPI was, unfortunately, not ascertained. Even 88453, with indubitable hypothyroidism, seemed to be able to raise SPI under the stimulus of pregnancy.

B90834, born in 1924, in 1940 was given up to 0.30 gm. of thyroid daily for obesity. This dose was later reduced to 0.12 gm., and in 1945 discontinued entirely for eight months, without noticeable effects. Thyroid was again resumed in a dose of 0.09 gm. because the basal metabolism was −16 per cent, with the idea that

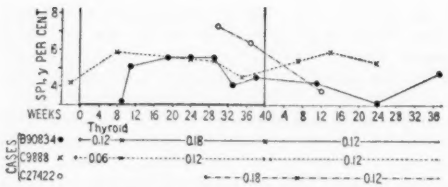

FIG. 7. THE COURSE OF SPI OF THREE QUESTIONABLY HYPOTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1.

it might promote conception. In 1947 she had a normal delivery after an uneventful pregnancy. November 3 of the same year her basal metabolism was −17 per cent. December 23 thyroid was discontinued. January 13, 1948, she had a prolonged menstrual period at the normal interval. Because a spontaneous abortion was suspected, she was ordered to resume 0.12 gm. of thyroid daily, despite the absence of evidences of hypothyroidism. On several occasions after this, because of irregular bleeding, pregnancy was suspected, but the Ascheim-Zondek test remained consistently negative. March 3 the dose of thyroid was increased to 0.18 gm., but within a month was again reduced to 0.12 gm. and finally, on October 18, was stopped entirely. The interruption coincided with her last menstrual period. December 20, when the first SPI was 3.2 γ per cent, she had been pregnant for nine weeks and had been taking 0.12 gm. of thyroid for five weeks. The course, beginning with this point, is illustrated in Figure 7.

C9888 was born in 1927. In 1940, because she lacked vigor, was somewhat somnolent and had oily skin, a basal metabolism was taken and found to be low. In 1942 her condition appeared to improve when she took 0.06 gm. of thyroid daily. In January, 1947, just before marriage, she discontinued medication with no other effect than slight retardation. January 22, 1948, she became pregnant. Her SPI two weeks earlier was 4.2 γ per cent, basal metabolism −16 per cent. Her course, beginning with this point, is illustrated in Figure 7.

C27422, born in 1920, in 1947, because of lassitude, infertility and a basal metabolism of −23 per cent, was given 0.12 to 0.18 gm. of thyroid daily. She noticed little change in her symptoms, but her basal metabolism rose to −5 per cent, to fall again to −17 per cent when she discontinued medication. She, therefore, resumed thyroid, taking from 0.03 to 0.12 gm. daily.

She became pregnant February 26, 1949. May 31, since she had no signs of thyroid disorder, the thyroid was discontinued. June 27, because her basal metabolism was −19 per cent, she was given 0.09 gm. of thyroid daily, which was progressively increased until on September 8 it was 0.18 gm. September 26, at the end of 30 weeks of pregnancy, SPI was 7.3 γ per cent, pulse rate 72. The course, beginning with this point, is illustrated in Figure 7.

The three patients in Figure 7 at one time or another received thyroid for presumptive hypothyroidism, although it is doubtful whether this diagnosis was justified. SPI of B90834 and C9888, on moderate doses of thyroid, rose in the early months of pregnancy to concentrations of 5.6 to 5.9 γ per cent, slightly lower than those usual in pregnancy, falling in the later part of pregnancy without change of therapy. The course of C27422 can not be distinguished in any respect from that of a normal pregnant woman. The trio emphasized what was brought out in an earlier paper (3), the ability of the pregnant woman to dispose of thyroid hormone.

A16616, born in 1907, on June 24, 1942, when she was six weeks pregnant, though free from symptoms, was found to have a palpable thyroid gland, slight exophthalmos, a pulse rate of 92, basal metabolism + 35 per cent and SPI 11.2 γ per cent. Her course, beginning at this point, is illustrated in Figure 8. She was followed altogether through three pregnancies and the intervals between them, receiving variable, sometimes intermittent, doses of Lugol's solution. Her course throughout resembled that of a normal woman. There were never clinical manifestations of hyperthyroidism and the pulse rate did not exceed 80.

C15718, born in 1926, in 1945 noted enlargement of her thyroid gland. In 1946 she had a normal pregnancy. When seen September 2, 1948, she had a moderate, diffuse enlargement of the thyroid, rather dry hair, a pulse rate of 68, basal metabolism − 22 per cent, SPI 3.8 γ per cent and serum cholesterol 197 mg. per cent. Her course, beginning with this point, is illustrated in Figure 8. There was nothing unusual about her course until 14 weeks after delivery when she was found to be gaining weight rather rapidly, her pulse rate was only 52 and she complained of pains in the upper part of her chest and feeling bloated after meals. One month later her weight had increased further, her skin had become dry and her face and hands seemed puffy. Serum cholesterol proved to be 358 mg. per cent (unfortunately the tube containing the specimen for determination of SPI was broken). She was given at once 0.12 gm. of thyroid, which was increased to 0.18 gm. daily four weeks later, because her symptoms had not been entirely allayed. April 10, 1950, two months after the thyroid was begun, SPI was 6.3 γ per cent, serum cholesterol 135 mg. per cent, pulse rate 70, weight normal, and she felt and appeared euthyroid.

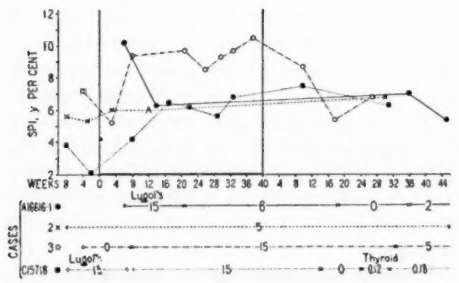

FIG. 8. THE COURSE OF SPI OF TWO QUESTIONABLY HYPERTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1. Case A16616 was followed through three pregnancies. The figures for therapy refer to drops of Lugol's solution except at the end of the course of Case C15718 when thyroid was substituted.

These two cases illustrated in Figure 8 are quite anomalous. There can be little doubt that A16616 had hyperthyroidism when she was first seen. Both SPI and evidences of hyperthyroidism subsided under the influence of Lugol's solution alone. Thereafter she ran a clinically almost euthyroid course in and out of pregnancy, with and without Lugol's solution in variable doses. On one occasion, after the third delivery, moderate signs of overactivity of the thyroid appeared with SPI 8.0 γ per cent, while she was receiving 5 drops of Lugol's solution daily. These subsided without change of therapy, SPI falling to 5.5 γ per cent, and subsequently to 3.4 γ per cent while she was taking no medication. The basal metabolism also was most capricious, usually above normal, with no apparent relation to SPI or clinical condition. This is a most abnormal pattern of behavior. Whether it indicated fluctuant thyroid activity it is impossible to say. Whatever its significance may have been, in pregnancy SPI followed the course expected in a euthyroid subject.

Case C15718 was given Lugol's solution in behalf of a goiter only. Shortly thereafter, just before pregnancy, SPI fell to 2.1 γ per cent, a concentration indicative of hypothyroidism. Nevertheless, it rose in pregnancy and remained elevated until 14 weeks after a normal delivery, when she suddenly developed hypothyroidism. It is possible that she was entering this state before pregnancy. In this case it must be inferred that the stimulus of pregnancy was able to elicit a response from the failing gland.

DISCUSSION

Certain difficulties present themselves in the analysis of these data. Hyperthyroidism is inherently a variable disease, marked by remissions

and exacerbations. In many instances there was no opportunity to observe the behavior of the patients for extended periods outside of pregnancy. Sometimes only fragments of the courses during pregnancy could be studied. Often it was not feasible to make observations at frequent or consistent intervals. The most desirable intervals for observations, indeed, were not known. It has been pointed out that SPI fluctuates greatly during normal pregnancy (3). It was impossible to follow any consistent course of therapy with justice to the patients; the treatment had to be regulated in accordance with the presumptive needs of each patient. In the earlier phases of the study the peculiar behavior of SPI during pregnancy was unknown. Attempts were, therefore, made to keep SPI within normal, nonpregnant limits. These were, however, somewhat tempered by fear for the consequence to the fetus should thyroid function be too much depressed. Efforts to strike a golden mean were not entirely successful. Recognition of the facts about SPI in pregnancy only complicated the problem. The last purely objective measure of thyroid activity was removed. Both basal metabolism and serum lipids are variously affected by pregnancy and are not, therefore, reliable indices of thyroid function in this state. Although both were measured at intervals, reliance had to be placed chiefly on clinical appraisal of the patients' conditions.

In spite of these difficulties the case material is large and varied enough to warrant some tentative deductions. In most instances SPI during pregnancy in patients with thyroid disorders, as in euthyroid subjects, was greater than 6.0 γ per cent. In some instances, usually as a result of treatment, it fell below 6.0 γ per cent temporarily or did not reach this concentration until pregnancy had advanced to 16 weeks. Occasionally it declined in the terminal weeks of pregnancy. In only two patients, B90834 and C9888 (Figure 7), did it remain persistently below 6.0 γ per cent, and in these two it was above 5.0 γ per cent until 33 weeks. In general it tended to follow the course observed in normal pregnancy, rising in the course of the first 16 weeks, usually remaining elevated until delivery. The time of the initial rise varied from case to case and was frequently modified by treatment; but in cases with more than one pregnancy there was a definite tendency for the same pattern of behavior to recur despite variable treatment.

In patients with active hyperthyroidism the SPI curves tended to lie at higher concentrations than the curves of euthyroid pregnant patients. Just as the latter tolerate in pregnancy concentrations of SPI that in nonpregnant women would be indicative of hyperthyroidism, so the hyperthyroid patient appeared to tolerate still higher values without symptoms. It was not possible to demonstrate this in all the subjects because some, for example B50050, when not pregnant, on occasions had unusually high SPI without hyperthyroidism. In the hyperthyroid subjects, however, this combination occurred with far greater frequency. Only two patients, C19922 and C24644, had symptoms suggestive of persistent hyperthyroidism during pregnancy with SPI under 10 γ per cent, and in these two the signs were equivocal. This does not imply that the tolerance of the hyperthyroid to high SPI is actually greater than that of the euthyroid subject because the SPI of the latter is not easily raised to the concentrations encountered in the hyperthyroid subjects. It merely indicates that in pregnancy higher SPI can be tolerated even if this arises from overactivity of the thyroid gland. This is circumstantial evidence that the increment in pregnancy is composed of thyroid hormone. The ability of the pregnant woman to dispose of exogenous hormone was demonstrated in the preceding paper. It is also evident in the three patients whose courses are shown in Figure 7.

In those patients in which antithyroid treatment was kept unchanged or was reduced during pregnancy, the course of SPI and symptoms, especially the latter, suggest that during pregnancy the requirement for antithyroid therapy diminishes. This might be interpreted as an indication that the tolerance for thyroid hormone is increased, a view that is in keeping with the traditional concept that the activity of the thyroid gland increases during pregnancy.

A further indication that the tolerance for thyroid is increased and the need for therapy diminished during pregnancy is found in the sudden rises of SPI associated with outbursts of hyperthyroidism encountered after delivery in a large proportion of the cases, especially the flagrant outbursts

associated with high peaks of SPI. These were encountered on four occasions in nine patients with definite hyperthyroidism. The three patients in whom they occurred were all followed for long periods without other similar incidents. All the peaks were noted within the narrow interval of 10 to 12 weeks after delivery. Since they appeared to be wholly or partly self-terminative and of short duration, they might have been discovered more often if observations postpartum had been made with greater frequency. Lesser rises of SPI associated with hyperthyroidism were noted after delivery in several other instances. These rises associated with hyperthyroidism differ from the persistently high values immediately after delivery, which are symptomless. SPI does not always fall immediately after delivery in hyperthyroid patients any more than it does in normal subjects (3); but may stay elevated for some time. These elevations, like those after normal pregnancy, are not associated with signs of hyperthyroidism. The tolerance to SPI is not coterminal with pregnancy but outlasts it by an appreciable interval. Both tolerance and interval may be greater in the thyroid patient. But, if the rise persists beyond a certain time, symptoms appear. The SPI of C38870 (Figure 5), for instance, rose to 15.9 γ per cent 11 days after delivery without evidences of hyperthyroidism, but six weeks later with the same concentration of SPI she had distinct symptoms.

The tendency for SPI to rise during pregnancy was evident even in the patients with hypothyroidism. This might suggest that the high SPI signified not increased secretion and utilization, but some block to the utilization of thyroid hormone. This interpretation appears unlikely, first of all because of the tolerance for exogenous thyroid evinced by euthyroid pregnant women (3). The lesser need for antithyroid therapy would also argue against such an hypothesis, unless it be also presumed that the secretory activity of the thyroid is decreased during pregnancy, which would make it difficult to explain the high values of SPI. The rises after delivery would be equally unaccountable on this basis. It would also be necessary on the diminished utilization theory to hypothesize that the tissues required less thyroid during pregnancy, else the hypothyroid patients should have sunk into myxedema as SPI rose. The SPI of case B82863 (Figure 1) shot up with the appearance of active hyperthyroidism after propylthiouracil had been discontinued for only two weeks, descending with equal speed when the previous dose of drug had been resumed. This rapid reaction indicates that secretion, utilization and sensitivity are all active. It has been remarked that in the authentic hypothyroid, 88453 (Figure 6), the low SPI found at 15 weeks followed abstention from medication for only one week. If it be assumed that it had previously been as high as it later was on the original dose of 0.12 gm., this would suggest an unusually rapid deterioration. C18328, in the same figure, appeared to utilize increasing amounts of thyroid in the later part of pregnancy. It is probable that clinical hypothyroidism in many cases is not a total, but a partial, deficiency. In some cases, indeed, it may represent only the absence of the normal stimulus to thyroid activity. It is conceivable that the stimulus of pregnancy may break down this inhibition or arouse the residual thyroid tissue to activity. In this connection the response of C15718 (Figure 8) is most suggestive.

In the preceding paper (3) it was pointed out that the SPI's of women receiving exogenous thyroid could remain low without compromising pregnancy. In the present series persistently low SPI was encountered only in patients who were receiving exogenous thyroid.

The argument thus far has presupposed that the increment of SPI in pregnancy is of the same composition as the preexisting SPI, chiefly thyroid hormone. This presumption is open to challenge. Danowski and associates (5) found that extraction of the serum with butanol by the method of Taurog and Chaikoff (6) yielded values in pregnancy that did not differ from SPI more than the values by the two methods differed in the sera of normal individuals. Similar results have been obtained by us in a number of sera (7). In this respect, at least the increment in pregnancy behaves like thyroxine. In addition, the reaction of euthyroid pregnant women to thyroid and the behavior of the patients with thyroid disorders presented in this paper are more easily explained on the hypothesis that the increment of SPI does consist of thyroid hormone.

SUMMARY AND CONCLUSIONS

The clinical course and the SPI during pregnancies of 16 patients with disorders of thyroid function have been analyzed. Treatment was limited to the use of thiourea, propylthiouracil, Lugol's solution and dried thyroid. The hyperthyroid patients became and remained euthyroid with concentrations of SPI characteristic of normal pregnancy, distinctly above those of normal nonpregnant women. There is some evidence that they were able to tolerate in pregnancy concentrations greater than 10 γ per cent, the highest value observed in euthyroid pregnant subjects. The data suggest that the requirements for antithyroid drugs may diminish during pregnancy and increase after delivery.

The implications of these phenomena with respect to the nature and significance of the high SPI of pregnancy are discussed.

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THE ANEMIA OF INFECTION. XIII. STUDIES ON EXPERIMENTALLY PRODUCED ACUTE HYPOFERREMIA IN DOGS AND THE RELATIONSHIP OF THE ADRENAL CORTEX TO HYPOFERREMIA [1]

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Hypoferremia is one of the most consistent features of the anemia associated with chronic infections in man (1). By what mechanism infection produces hypoferremia is not known. It has been reported previously that in dogs an acute, transient, hypoferremia of pronounced degree develops following the experimental production of an infected (staphylococcus) or a sterile (turpentine) intramuscular abscess (2). Since sterile abscesses produce hypoferremia, it is evident that bacteria and their products are not essential for the reaction. It has, furthermore, been possible to demonstrate that in dogs the intravenous injection of colloidal thorium dioxide is associated with a pronounced hyperferremia and that following the injection of thorium the hypoferremia-producing effect of turpentine does not develop (3). It would appear, therefore, that in infection the reticulo-endothelial system is stimulated to store iron.

By the use of intravenously injected radioactive iron, it has been shown that the utilization of iron for hemoglobin synthesis is impaired in the presence of infection (4, 5). Furthermore, the administration of extremely large amounts of iron intravenously in man fails to relieve the anemia and the hypoferremia persists (6). There is a reduced uptake of iron by the heme of the red blood corpuscles and the iron is diverted mainly to the liver and spleen (7). This would seem to indicate that whatever the mechanism whereby the low level of iron in the plasma is produced, it is extremely powerful and cannot be easily overcome.

The purpose of the experiments reported here was to study further the mechanism by which inflammation produces hypoferremia in dogs.

METHODS

In these studies approximately 200 male and female mongrel dogs weighing between 10 and 15 kg. were used. Of these 65 were adrenalectomized. The animals were fed Purina Dog Chow and allowed water *ad libitum* except during the experimental procedures, at which time they were fasted.

Adrenalectomies were performed in one stage. The dogs were anesthetized with 5 per cent nembutal intravenously, and the adrenal glands were exposed and removed through the transabdominal approach. Sterile precautions were used throughout the procedure.

At the beginning of the operation the dogs were given an intramuscular injection of 5 ml. of aqueous or 0.5 ml. of lipo-adrenal cortical extract, and a similar dose was given on its completion. Desoxycorticosterone acetate was found to be of little supportive value at this stage and dogs supported with this steroid during the operative procedure invariably failed to survive adrenalectomy. During the operation the animals received an intravenous infusion of 250–500 ml. of 5 per cent dextrose in normal saline. Dogs of medium size survived adrenalectomy best. For the first three days following the operation, the dogs were given 0.5 to 1 ml. of lipo-adrenal or 5 ml. of aqueous adrenal cortical extract intramuscularly daily. After this they were given 0.5 ml. of lipo-adrenal cortical extract every two or three days. No extract was given for at least 48 hours before the start of an experimental procedure. At one period during these experiments, the supply of adrenal cortical extract was limited and the dogs were maintained postoperatively on 1 mg. desoxycorticosterone acetate intramuscularly every day.

For the first week postoperatively the dogs were fed a diet of rolled oats to which sodium chloride, sucrose and milk were added. Thereafter, they were given a Purina Dog Chow diet with a 0.9 per cent solution of sodium chloride to drink.

All glassware used for the collection of blood samples and for the determination of plasma iron was carefully cleansed with acid and washed three times with water double distilled in an all glass distillation apparatus. Ve-

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nous blood samples were drawn into all glass syringes and placed in 15 ml. centrifuge tubes to which two drops of 20 per cent potassium oxalate had been added previously. The plasma was then separated by centrifugation and the iron estimation made by a modification (8) of the method of Barkan and Walker (9), the Beckman spectrophotometer being used.

To estimate the errors introduced by differences in venipuncture samples, the handling of the blood and the iron estimations themselves, four consecutive samples were drawn in four separate venipunctures from each of five dogs. The time which elapsed from the taking of the first sample and the last did not exceed five minutes. Dogs with a wide range of plasma iron levels were chosen for this experiment to see if the level affected the degree of variability within a single dog. Table I gives the results obtained. The standard error of a single determination was calculated for these results

$$\left(\text{S.D.} = \sqrt{\frac{\Sigma(X - \overline{X}_a)^2}{n - 1}}\right)$$

and was found to be ± 3.7 μg. per cent.

In the earlier experiments, samples were taken for iron estimation immediately before an experimental procedure and at intervals throughout the 24 hour period. Additional samples were then taken at 48, 72 and 96 hours. In subsequent experiments samples for iron determinations were restricted to one immediately before an experimental procedure and one at six, eight and 24 hours following this. Portions of these samples, collected in a mixture of ammonium and potassium oxalate, were used for the determination of the volume of packed red blood cells and for cell counts. Total leukocyte counts, differential blood cell counts and absolute eosinophil counts were made immediately before an experimental procedure and at two, four and six hours following it. These were carried out in the hope that they might serve as indices of adrenocortical activity (10–12). Total white and differential blood cell counts were carried out by routine methods. The differential cell determinations were made on duplicate cover slip preparations. 200–500 cells were examined for each count. Absolute eosinophil counts were made by the phloxine propylene glycol method (13). Duplicate counts were made in 16 squares of a Fuchs' Rosenthal counting chamber of 0.2 mm. depth.

TABLE I

Variations in the plasma iron in four consecutive samples from five dogs

Values are in μg. per cent

Sample	Dog number				
	1	2	3	4	5
1	74	56	59	292	112
2	71	59	56	290	107
3	78	55	64	290	112
4	73	50	68	290	102

TABLE II

Variations in the total white blood cell counts per c.mm. in four consecutive samples from six dogs

Sample	Dog number					
	1	2	3	4	5	6
1	13,400	9,600	8,350	6,800	7,900	23,100
2	14,000	9,600	9,000	6,700	6,250	23,750
3	13,900	8,800	9,250	8,300	8,200	23,300
4	14,250	—	7,450	8,400	8,750	25,300

To determine the variations which might be attributed to technical factors in making the absolute white cell and eosinophil counts, four consecutive samples were drawn into double oxalate with four separate venipunctures from each of six dogs. The time which elapsed from the taking of the first sample and the final one did not exceed five minutes. The results obtained are given in Tables II and III.

It is obviously not possible to compare absolute values for white blood cell counts of one animal with those of another. However, if the changes are expressed in proportion to the original count, it is possible to compare these percentage changes from animal to animal. The standard error of a single determination of the total white blood cell count has been calculated from the data in Table II and found to be ± 9.1 per cent.

The standard error of a single determination of the absolute eosinophil count, calculated from the results in Table III gives a value ± 10.3 per cent.

In the differential cell count the white blood cells were divided into four types: polymorphonuclear cells, eosinophils, lymphocytes and monocytes. The absolute number of each type of cell was determined by multiplying the per cent of each cell in the differential count by the total number of white blood cells. The absolute numbers of each type of cell subsequent to an experimental procedure were then expressed as a percentage of the initial level. The observed changes in the monocyte counts appeared to have no definite trend and these have been excluded from the results.

All data have been treated statistically where possible. The standard error of the mean has been calculated and is given with mean values. The t test has been used to estimate the significance of differences between groups.

TABLE III

Variations in the absolute eosinophil counts per c.mm. in four consecutive samples from six dogs

Sample	Dog number					
	1	2	3	4	5	6
1	294	169	56	406	506	500
2	284	120	44	447	494	547
3	288	138	50	383	444	512
4	278	—	38	413	525	531

RESULTS

Studies in Intact Dogs

The effect of taking blood samples and of a single injection of normal saline on the plasma iron. To evaluate the part played by the taking of blood samples on variations of the plasma iron, and also to measure the variations with the time of day, five dogs were bled at regular intervals during the day and three dogs were given 5.0 ml. saline intramuscularly and also bled after the initial sample had been drawn (Figures 1 and 2). There was no difference in the variations observed in those dogs which received no treatment and in those which received the injection of saline. Both groups may therefore be considered as belonging to the same population. In seven dogs there was some lowering of the plasma iron level between six and eight hours after the initial sample, the mean per cent decrease for the group being 24 ± 6. The iron level of one dog rose during the day. The levels of all eight dogs returned to their initial value at the end of 24 hours. It is interesting to note (Figure 1) that the per cent decrease in the plasma iron of four dogs was approximately 50 and that in the other four the range was from 0 to -17, the latter values being for the most part within the variations to be expected with the experimental methods used. This point and the correlation between these changes and those observed in the cellular elements of the blood will be dealt with in greater detail in the discussion.

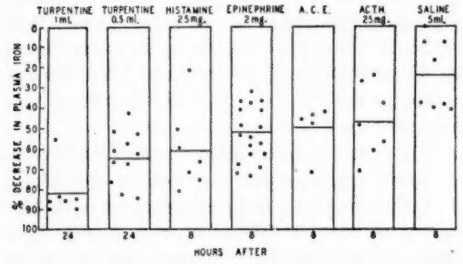

FIG. 1. THE MAXIMAL HYPOFERREMIA-PRODUCING EFFECT IN THE DOG OF THE INTRAMUSCULAR INJECTION OF 1 ML. AND 0.5 ML. OF TURPENTINE; 25 MG. OF HISTAMINE; 2 MG. OF EPINEPHRINE; ADRENAL CORTICAL EXTRACT; 25 MG. OF ACTH; AND OF MILD "STRESS" AS REPRESENTED BY THE INJECTION OF 5 ML. SALINE

Each point represents the maximal decrease in the plasma iron of a single dog.

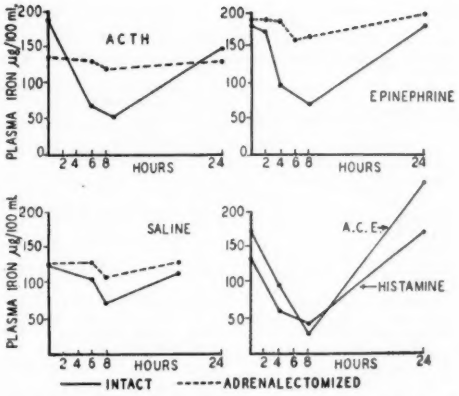

FIG. 2. REPRESENTATIVE EXAMPLES OF THE CHANGES IN THE PLASMA IRON PRODUCED BY THE INTRAMUSCULAR INJECTION OF 25 MG. OF ACTH; 2 MG. OF EPINEPHRINE; 5.0 ML. OF 0.9 PER CENT SALINE, IN INTACT (CONTINUOUS LINE) AND ADRENALECTOMIZED (DOTTED LINE) DOGS, AND BY THE INTRAMUSCULAR INJECTION OF 25 MG. OF HISTAMINE AND THE INTRAPERITONEAL INJECTION OF 120 ML. OF AQUEOUS ADRENAL CORTICAL EXTRACT IN INTACT DOGS

The effect of turpentine on the plasma iron. In earlier studies it was shown that the intramuscular injection of 5 or 10 ml. of turpentine (rectified oil of turpentine, Rexall, USP) was followed by characteristic changes in the plasma iron (*2*, *3*). The level of iron was significantly lowered in 24 hours and reached its lowest values within 24 to 48 hours, the mean per cent decrease being 78 ± 3. This hypoferremic phase was followed by a hyperferremic phase of brief duration, after which the plasma iron returned to normal. Similar results have been obtained in this investigation following the injection of 1.0 ml. (in seven dogs) and 0.5 ml. (in 11 dogs) of turpentine. After 24 hours these amounts produced mean decreases in the plasma iron level of 82 ± 5 and 65 ± 4 per cent, respectively (Table IV). The percentage decrease following the injection of 1.0 ml. and of 0.5 ml. of turpentine for each individual dog is plotted in Figure 1.

The effect of histamine on the plasma iron. A single intramuscular injection of 25 mg. of histamine in aqueous solution was associated with

TABLE IV

The hypoferremic effect of a variety of agents in intact and adrenalectomized dogs

Substance injected	Group	No. of dogs	Initial P.I. μg. per cent			Lowest subsequent P.I. μg. per cent			Decrease in P.I. μg. per cent			Per cent decrease in P.I.			Time after injection
			Mean	S.E.	Range	Mean	S.E.	Range	Mean	S.E.	Range	Mean	S.E.	Range	Hours
Histamine 25 mg.	Intact	7	149	±17	102–234	55	± 8	20– 83	94	±18	23–178	61	±8	22–81	8
Adrenal cortical extract	Intact	5	145	±17	103–195	72	±11	48–113	75	±14	47–125	50	±5	42–72	8
Turpentine 5.0 ml.	Intact	10	174	±11	110–229	38	± 2	22– 45	136	±12	65–194	78	±3	59–89	24
Turpentine 1.0 ml.	Intact	7	169	±21	64–253	25	± 1	20– 30	145	±22	36–228	82	±5	56–90	24
Turpentine 0.5 ml.	Intact	11	130	±14	63–216	43	± 5	23– 91	86	±12	27–174	65	±4	43–85	24
Turpentine 0.5 ml.	Intact	11	130	±14	63–216	89	±14	36–177	41	±10	0– 93	32	±7	0–72	8
	Adect.	5	114	±20	66–154	91	±20	46–147	24	± 4	12– 35	22	±5	5–30	8
Epinephrine 2.0 mg.	Intact	18	136	± 9	53–190	61	± 5	33–119	75	± 8	20–126	53	±3	32–74	8
	Adect.	9	113	±15	38–191	85	±14	28–160	29	± 7	0–58	26	±6	0–47	8
ACTH 25 mg.	Intact	7	160	±23	84–274	79	± 8	55–117	82	±20	23–157	47	±7	24–71	8
	Adect.	6	107	±13	63–145	99	±11	51–122	11	± 4	0–26	10	±3	0–19	8
Saline 5.0 ml.	Intact	8	127	±14	79–195	101	±13	60–157	32	±11	0–76	24	±6	0–41	8
	Adect.	11	68	± 8	41–124	70	±17	48–115	4	± 2	0–15	5	±3	0–24	8

changes in the plasma iron similar to those which followed the injection of turpentine. They differed from those which followed turpentine in that the maximum hypoferremia occurred within six to eight hours instead of 24 to 48; at 24 hours after histamine injection the iron level returned to its previous level or was in the brief hyperferremic phase. The mean per cent decrease in the plasma iron of seven dogs at six to eight hours after histamine injection was 61 ± 8 (Table IV). Figure 2 is a representative example of the change in the plasma iron level following the injection of histamine while the decreases for each individual dog are given in Figure 1.

Repeated intramuscular injections of 20 mg. of histamine in aqueous solution were given to two dogs over three days. Both dogs received an injection of histamine at 9:00 a.m. and at 4:00 p.m. on each of three days. The first injection of histamine was followed by a marked decrease in the plasma iron level of the two dogs. Twenty-four hours after the initial injection of histamine the level showed a tendency to revert to its previous value. The level of iron at 48 hours, with the one exception, remained lower than the value prior to the first injection of histamine. At 65 hours after the last injection the levels of iron in both dogs were considerably greater than the initial values and reverted to the initial levels some 24 to 48 hours later. These results are shown in Figure 3. During the period of the repeated injections of histamine the volume of packed red cells of both dogs remained constant.

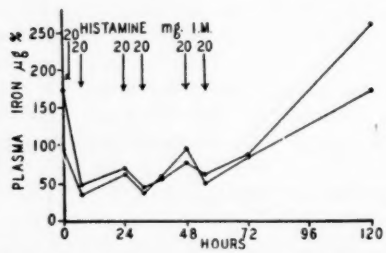

FIG. 3. THE EFFECTS OF THE REPEATED INTRAMUSCULAR INJECTION OF 20 MG. OF HISTAMINE ON THE PLASMA IRON OF TWO DOGS

The effect of epinephrine on the plasma iron. A single intramuscular injection of 2.0 ml. of 1:1,000 epinephrine in aqueous solution to 10 dogs and of 2.0 ml. of 1:500 epinephrine in oil to eight dogs was followed by a hypoferremia similar to that resulting from histamine (Figure 2). Maximal hypoferremia occurred between six and eight hours after the injection. The plasma iron after 24 hours returned to its pre-injection level or higher. There was no difference in the time, and no correlation between the extent of the alteration of the plasma iron produced by the two doses and

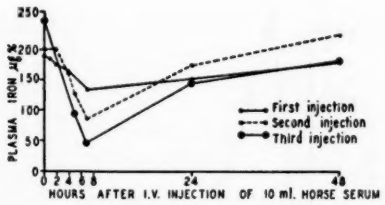

FIG. 4. THE CHANGES IN THE LEVEL OF PLASMA IRON OF A DOG FOLLOWING THE PRODUCTION OF ANAPHYLACTIC SHOCK BY THE INTRAVENOUS INJECTION OF HORSE SERUM

the preparations of epinephrine used. The mean per cent decrease in the plasma iron level was 53 ± 3 (Table IV). The decrease for each dog is plotted in Figure 1.

The effect of anaphylactic shock on the plasma iron. The single intravenous injection of 10 ml. of horse serum to a dog, which two weeks previously had received 10 ml. of horse serum intravenously, was followed by a 20 per cent decrease in the plasma iron at seven hours. The plasma iron level returned to its pre-injection level within 48 hours. Within a few minutes after the injection of the horse serum the dog showed considerable reaction including urination and defecation. Fourteen days later a second intravenous injection of 10 ml. of horse serum was given to the same dog. This was followed by a severe reaction, and seven hours later the plasma iron decreased 58 per cent below its initial level. At 24 to 48 hours the concentration of plasma iron had returned toward the pre-injection level. Seven weeks later the same dog was given a third intravenous injection of 10 ml. horse serum. This time the dog showed only a slight reaction but seven hours following the injection the level of iron had decreased 80 per cent, to return to its pre-injection level at 48 hours. The effects of the three injections of horse serum are shown in Figure 4.

The effect of fracture of the femur on the plasma iron. Two dogs were anesthetized with 5.0 per cent sodium pentobarbital. It had been found in nine dogs that sodium pentobarbital anesthesia had an effect on the plasma iron identical with that of mild stress or the intramuscular injection of saline, the mean per cent decrease at six to eight hours being 22 ± 10. Following fracture of the femur under anesthesia, the plasma iron level decreased 39 and 33 per cent, respectively, in the two dogs at seven hours but maximal hypoferremia occurred at 24 hours, at which time the decrease was 75 and 46 per cent, respectively. The level of plasma iron then rose again at 48 hours and at 72 hours was approaching its pre-fracture level (Figure 5). Thus in their temporal relationships the alterations in the plasma iron level produced by fracture resemble those produced by turpentine, rather than those produced by histamine, epinephrine or mild stress.

The effect of miscellaneous "stresses" on the plasma iron. The single intramuscular injection of 5 ml. of formaldehyde in one dog was followed in seven hours by a 46 per cent decrease in the plasma iron level. Four dogs were kept for eight hours at − 20° C. The stress of cold had no consistent effect on the level of plasma iron.

The effect of adrenal cortical extract on the plasma iron. Five dogs were given single injections of adrenal cortical extract. Two received 30 ml. of lipo-adrenal and one 60 ml. of aqueous adrenal cortical extract intramuscularly. The remaining two received 40 ml. and 120 ml. of aqueous adrenal cortical extract intraperitoneally, the alcohol having been removed previously. The variations in the doses and routes of administration used did not produce discernible differences in the variations in the plasma iron level. As with histamine and epinephrine maximum hypoferremia occurred between six and eight hours after the injection, the mean per cent decrease for the five dogs being 50 ± 5 (Table IV). The level of iron returned to its previous value or higher at 24

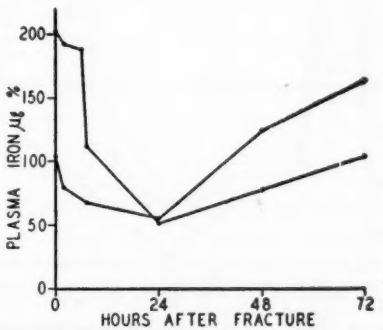

FIG. 5. THE EFFECTS OF FRACTURE OF THE FEMUR ON THE LEVEL OF PLASMA IRON IN TWO DOGS

hours. A typical response is shown in Figure 2 and the individual decreases for the five dogs are plotted in Figure 1.

The effect of adrenocorticotrophic hormone (ACTH) on the plasma iron. A single injection of ACTH was given to five dogs. Of these, two received the injection intramuscularly and three intravenously. The dose for all five was equivalent to 25 mg. of Armour's standard (LA–1–A) dissolved in 5 ml. normal saline. An additional two dogs were given a dose equivalent to 60 mg. of Armour's standard (LA–1–A) given intravenously in five divided doses at 30 minute intervals for two hours. The changes in the plasma iron level were similar for all seven dogs and they will be considered as a group. There was a maximal drop in the plasma iron of all dogs between six and eight hours after the last injection of ACTH, the mean per cent decrease being 47 ± 7 (Figure 1). This was followed, as with histamine, epinephrine and adrenal cortical extract, by a return of the iron to its pre-injection level or to a slightly higher level after 24 hours (Figure 2).

The effect of Dibenamine (N, N-Dibenzyl-β-Chloroethyl-Amine-Hydrochloride) on the plasma iron. Three dogs were given a single intravenous injection of 20 mg. per kg. of body weight of Dibenamine in 30 ml. of 0.9 per cent solution of sodium chloride. The animals showed signs of central excitation with tremors and vomiting within 30 minutes. Another three dogs received the same dose of Dibenamine in a 200 ml. infusion of 0.9 per cent solution of sodium chloride over 10 minutes and showed some excitation but this had no untoward results. The effects of the injection of Dibenamine on the level of plasma iron were identical for both groups. Within one hour following the injection, the level of the plasma iron began to rise and a maximum increase was reached at approximately two hours after the injection of the drug, the mean per cent increase over the initial iron level being 59 ± 4. At six hours following the injection the level of iron had fallen towards the pre-injection level and at eight hours it was below this level, with a mean per cent decrease of 30 ± 3. At 24 hours the hypoferremia was maximal, the mean per cent decrease from the initial iron level being 60 ± 5; this was followed by a return towards the control level at 48 hours. These results are shown in Figure 6.

Following the intravenous administration of 5 mg. per kg. of body weight of Dibenamine in 200 ml. of 0.9 per cent solution of sodium chloride to three dogs there were no demonstrable signs of central excitation. The plasma iron level rose in all three dogs within the first two hours following

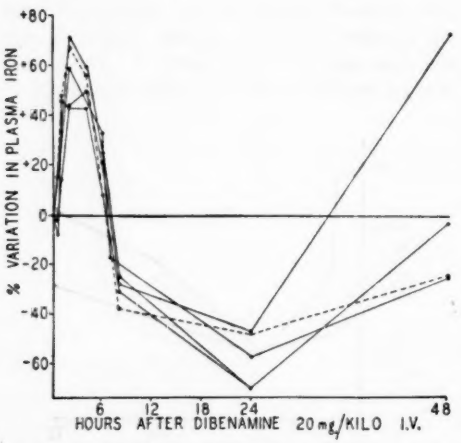

FIG. 6. THE CHANGES IN THE PLASMA IRON OF FIVE DOGS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 20 MG. PER KG. OF DIBENAMINE

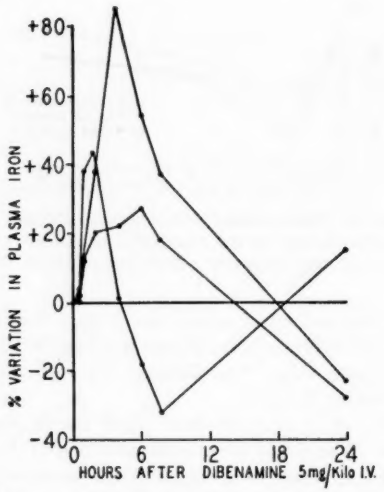

FIG. 7. THE CHANGES IN THE PLASMA IRON OF THREE DOGS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 5 MG. PER KG. OF DIBENAMINE

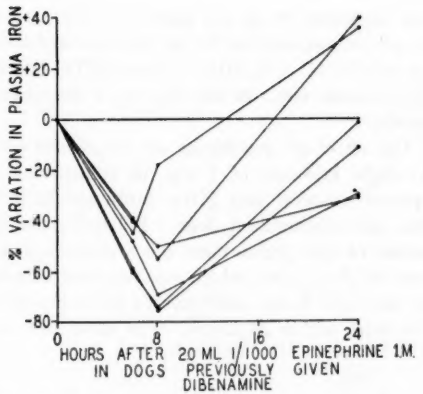

FIG. 8. THE CHANGES IN THE PLASMA IRON FOLLOWING THE INTRAMUSCULAR INJECTION OF 2 MG. OF EPINEPHRINE IN SIX DOGS PREVIOUSLY GIVEN DIBENAMINE

the injection but the hypoferremic phase which usually took place between six and eight hours after stress occurred only in one dog, the other two showing moderate hypoferremia at the 24 hour period (Figure 7). The differences in the variations in the plasma iron level produced by the two doses of Dibenamine will be correlated with the changes noted in the cells. The significance of this correlation is considered in the discussion.

The effect of epinephrine following the administration of Dibenamine on the plasma iron level. A single intramuscular injection of 2 mg. of epinephrine in aqueous solution was given to six dogs. Three of these had previously received 20 mg. per kg. of body weight of Dibenamine, while the other three received 5 mg. per kg. of body weight of Dibenamine. The epinephrine was given to one dog from each dosage group at 24, 48 and 72 hours after the administration of Dibenamine. In all six dogs the injection of epinephrine was followed by a decrease in the plasma iron level which was maximal between six and eight hours after the injections. At 24 hours the iron level showed a tendency to revert to its pre-injection value (Figure 8). The mean per cent decrease in the plasma iron below the initial levels was 61 ± 5. This is very similar to the decrease following the injection of epinephrine in otherwise untreated intact dogs. Thus Dibenamine failed to block the hypoferremic effect of epinephrine.

Studies in Adrenalectomized Dogs

The effect of adrenalectomy on the plasma iron level. A minimum of one week was allowed to elapse following adrenalectomy before a dog was used in an experiment. The mean initial plasma iron levels were all lower in the adrenalectomized group than in the corresponding intact group (Table IV). However, in only one group was the decrease significant and this was in the mildly stressed group ($P = < .001$). The dogs in this group were maintained largely on desoxycorticosterone acetate and not on adrenal cortical extract.

This tendency to a lowering of the plasma iron level following adrenalectomy would have been more marked if several adrenalectomized dogs, whose pre-injection plasma iron levels were very low, had not been excluded from the results in order that the initial iron levels of intact and adrenalectomized groups might be more comparable.

The effect of mild stress on the plasma iron. Samples were taken from three adrenalectomized dogs at regular intervals throughout the day and eight adrenalectomized dogs were given 5 ml. of 0.9 per cent sodium chloride solution intramuscularly. The effect on the plasma iron level of all dogs was identical and, in contrast to the changes noted in intact dogs, the plasma iron levels showed no significant change (Figure 9). The maximum mean per cent decrease noted was 5 ± 3 which is

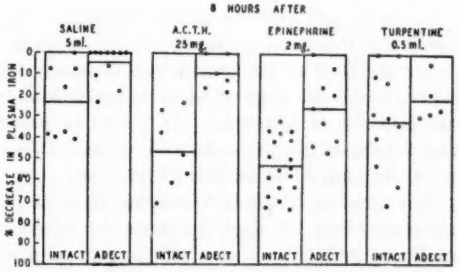

FIG. 9. THE MAXIMAL HYPOFERREMIA-PRODUCING EFFECT OF MILD STRESS (SALINE), OF THE INTRAMUSCULAR INJECTION OF 25 MG. OF ACTH, 2 MG. OF EPINEPHRINE AND 0.5 ML. OF TURPENTINE IN INTACT AND ADRENALECTOMIZED DOGS

Each point represents the maximal decrease in the plasma iron of an individual dog.

within the experimental error of the methods used. There was a significant difference between the intact and adrenalectomized dogs in the decrease in plasma iron in μg. per cent ($P = < .001$) and in the percentage decrease in the plasma iron following mild stress ($P = < .01$). Thus adrenalectomy abolished the hypoferremic effect of mild stress.

The effect of turpentine on the plasma iron. The intramuscular injection of 1 ml. of turpentine in two adrenalectomized dogs resulted in their death within six hours. The dose was therefore reduced to 0.5 ml. Five dogs survived this dose until eight hours after the injection but only two survived for 24 hours. Of these two survivors, the iron level of one showed a 48 per cent decrease from its pre-injection value, and the level of the other showed no change. The latter is the only dog in our series in which the intramuscular injection of turpentine did not produce hypoferremia (Figure 10). An additional adrenalectomized dog was given a single injection of 0.5 ml. of turpentine, this animal being supported throughout the 24 hour period with three intramuscular injections of 5 ml. of aqueous adrenal cortical extract. We had previously found, in two intact dogs, that the single intramuscular injection of 25 ml. of aqueous adrenal cortical extract was not sufficient to cause a lowering of the plasma iron. In this dog the injection of turpentine was followed by a fall in the plasma iron level at 24 hours, comparable with the mean per cent decrease obtained in the intact dogs. These results are shown in Figure 10.

It can be seen from the scatter diagram in Figure 9 that there was no significant difference between the level of the plasma iron of intact and adrenalectomized dogs at eight hours following the injection of turpentine. It should be noted that maximum hypoferremia with turpentine does not occur until 24 hours after its injection.

The effect of adrenocorticotrophic hormone on the plasma iron. A single intramuscular injection of 25 mg. ACTH in 5 ml. of 0.9 per cent solution of sodium chloride to six dogs was not followed by any significant change in the plasma iron level (Figure 9). The maximum mean per cent decrease was 10 ± 3 which again was within the experimental error of the methods used. There was a significant difference between the intact and adrenalectomized dogs in the decrease in the plasma iron expressed in μg. per cent ($P = .01$) and in the percentage decrease in the plasma iron following ACTH ($P = < .001$). Thus ACTH had no hypoferremic effect in the absence of the adrenal glands.

The effect of epinephrine on the plasma iron. A single injection of 2 mg. of epinephrine in aqueous solution was given intramuscularly to nine adrenalectomized dogs. Following the injection of epinephrine there was a decrease in the level of plasma iron which was maximal between six and eight hours and returned to its pre-injection level within 24 hours. The mean per cent decrease in plasma iron for the group was 26 ± 6 (Table IV). This decrease was significantly different from that obtained in the intact dog following epinephrine ($P = < .001$). This decrease was also significantly different from the effect of mild stress and saline in the adrenalectomized dogs ($P = < .01$). Thus epinephrine produced a significant hypoferremia in the absence of the adrenal cortex, but this was significantly less than that which was obtained in the intact dog. This is shown in Figure 9.

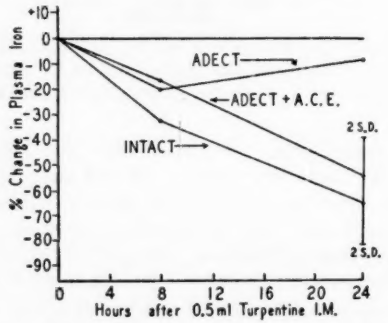

FIG. 10. THE MEAN PER CENT CHANGE IN THE LEVEL OF THE PLASMA IRON OF 11 INTACT DOGS FOLLOWING THE INTRAMUSCULAR INJECTION OF 0.5 ML. OF TURPENTINE, WITH A LINE INDICATING THE 95 PER CENT RANGE OF MAXIMAL HYPOFERREMIA AFTER 24 HOURS

The changes in the plasma iron of an adrenalectomized dog which survived the intramuscular injection of 0.5 ml. of turpentine and of another adrenalectomized dog which was supported with 15 ml. of adrenal cortical extract during this period are also shown.

The effect of adrenocorticotrophic hormone, colloidal thorium dioxide and adrenalectomy on the rate of the removal of colloidal saccharated oxide

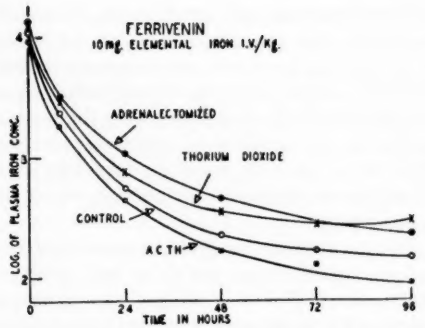

FIG. 11. THE RATE OF DISAPPEARANCE OF INTRAVENOUSLY ADMINISTERED SACCHARATED OXIDE OF IRON FROM THE PLASMA OF FOUR GROUPS OF DOGS; INTACT UNTREATED (CONTROL), INTACT PREVIOUSLY INJECTED WITH COLLOIDAL THORIUM DIOXIDE, INTACT AND GIVEN ACTH, AND ADRENALECTOMIZED

of iron from the plasma. An intravenous injection of saccharated oxide of iron (SKF brand) equivalent to 10 mg. of elemental iron per kg. of body weight was given to four groups of dogs. These consisted of an intact untreated group of four dogs; an intact group of four dogs given 10 mg. ACTH before the injection of the iron and at 6, 12, 24, 30 and 36 hours following its administration; an adrenalectomized group of three dogs; and an intact group of four dogs which had received intravenous injections of 2 ml. per kg. of body weight of colloidal thorium dioxide (Thorotrast, Heyden Company, New York City) for three consecutive days, the iron being given 24 hours after the last injection of the thorium. Blood samples for the estimation of plasma iron were drawn immediately before the injections of the intravenous iron and at 10 minutes, 8, 24, 48, 72 and 96 hours following it.

TABLE V

White blood cell counts per c.mm. in intact and adrenalectomized dogs

Cells	Intact		Adrenalectomized	
	No. of dogs	Mean ± S.D.	No. of dogs	Mean ± S.D.
Total	64	12,940±3,600	24	12,800±3,900
Neutrophils	59	9,300±3,000	24	8,000±2,650
Lymphocytes	59	2,528±1,160	24	3,100±1,460

In the four groups of dogs the iron was removed most rapidly from the plasma of the dogs given injections of ACTH and most slowly from the plasma of the adrenalectomized dogs. The rate of disappearance of iron from the plasma of the intact untreated dogs fell between these two groups, and that from the plasma of the dogs previously given thorium dioxide came between the rate of disappearance of iron from the intact untreated controls and the adrenalectomized dogs. These results are shown in Figure 11.

Four days after the administration of the saccharated oxide of iron, the intact dogs previously given thorium dioxide, were given a single intramuscular injection of 25 mg. of histamine. The mean per cent decrease in the plasma iron was 16 ± 10 which was significantly different from the 61 ± 8 per cent decrease obtained following the injection of histamine in intact untreated dogs ($P = < .01$).

Studies on the Leukocytes of Intact and Adrenalectomized Dogs Before and After the Injection of Various Substances

The mean cell counts in intact and adrenalectomized dogs before they were subjected to any experimental procedures are summarized in Table V.

TABLE VI

Maximum per cent changes in the level of the white blood cells following the injection of a variety of agents in intact and adrenalectomized dogs

Substance injected	Group	No. of dogs	WBC		PMN		Eosinophils		Lymphocytes	
			Mean±S.E.	Range	Mean±S.E.	Range	Mean±S.E.	Range	Mean±S.E.	Range
Turpentine 0.5 ml.	Intact.	10	+60 ±13	9–127	+ 83 ±16	38–187	−81 ±6	(−37)–(−100)	−42 ±6	(0)–(−69)
	Adect.	4	+32 ±11	3–56	+ 58 ±14	17–78	−38 ±7	(−21)–(−51)	+15 ±26	(+81)–(−28)
Epinephrine 2.0 mg.	Intact.	5	+74 ±17	32–118	+125 ±26	60–194	−80 ±10	(−47)–(−100)	−62 ±6	(−51)–(−81)
	Adect.	6	+15 ±4	0–28	+ 21 ±4	6–34	+19 ±10	(+97)–(−43)	+33 ±29	(+136)–(−55)
ACTH 25 mg.	Intact.	10	+58 ±9	0–104	+103 ±6	11–193	−84 ±6	(−37)–(−100)	−30 ±7	(0)–(−63)
	Adect.	6	+35 ±15	4–90	+ 37 ±15	2–100	+50 ±23	(+134)–(−40)	+21 ±26	(+131)–(−48)
Saline 5.0 ml.	Inact.	10	+29 ±10	0–94	+ 41 ±10	5–111	−30 ±10	(0)–(−77)	−21 ±5	(−1)–(−46)
	Adect.	6	+24 ±13	0–75	+ 23 ±12	0–79	+30 ±32	(+140)–(−67)	+36 ±21	(+97)–(−27)

The figures for the intact dog are in close agreement with those given by Gardner (14) in her most comprehensive review of the literature on the blood picture in the dog.

There was no difference between the total leukocyte count of intact as compared with adrenalectomized dogs. There was a tendency for the lymphocytes to be increased ($P = < .2$) and for the polymorphonuclear cells to be decreased ($P = < .5$) in the adrenalectomized dogs although these differences were not statistically significant.

The maximum percentage changes noted in the leukocytes following the intramuscular injection of turpentine, epinephrine, ACTH and saline in both intact and adrenalectomized dogs are summarized in Table VI.

Following the injection of turpentine, epinephrine and ACTH into intact dogs there was a rise in the total white blood cell and polymorphonuclear cell counts (maximal at six hours following the injection) and a significant decrease in the eosinophils (maximal at six hours) and in the lymphocytes (maximal at four hours). Following the injection of these agents in adrenalectomized dogs there was a tendency for the increases in the white blood cell and polymorphonuclear cell counts to be less than those obtained in the intact animals but these differences were only significant in the epinephrine group. Turpentine was the only agent which produced a significant decrease in eosinophils in the absence of the adrenal cortex. However, this decrease was significantly less than that obtained in intact dogs given turpentine ($P = < .01$). In contrast to the intact dogs, the lymphocytes of the adrenalectomized animals showed a tendency to increase rather than decrease following the administration of each of the three agents.

It is interesting to note in greater detail the effects of mild stress on the leukocytes of intact dogs. Of the eight intact dogs on which plasma iron estimations were also made, there were four in which the per cent decrease in iron was significant and four in which it was not. Of the four in which the iron showed a significant decrease, blood cell counts were made on three and the mean increase of the white blood cell and polymorphonuclear cell counts were 51 and 62 per cent. Of the four dogs in which the iron showed no significant change, cell counts were simultaneously taken on three and the mean per cent increase of the white blood cell and polymorphonuclear cell counts were 9 and 24, showing a direct correlation between the change in the level of plasma iron and changes in the level of the cell counts. Similarly, in the three dogs with a decrease in the level of plasma iron, the per cent fall in the eosinophils and lymphocytes averaged 71 and 20, respectively, and in the three dogs which did not show a change the per cent fall was 54 and 9, respectively.

Two hours following the intravenous injection in three dogs of 20 mg. per kg. of body weight of Dibenamine in 200 ml. of 0.9 per cent solution of sodium chloride, there was a marked hyperferremia and a mean per cent increase in the total white blood count and polymorphonuclear count of 15 ± 8 and 25 ± 11, respectively. Both the eosinophils and lymphocytes showed a decrease at this period of 24 ± 5 and 15 ± 13 per cent. The total leukocytes and polymorphonuclear cells increased to a maximum eight hours following Dibenamine at which time they showed a mean per cent increase of 65 ± 20 and 90 ± 25, over their initial counts. The greatest fall in the eosoinophil count was at six hours, when they showed a decrease of 71 ± 7 per cent, and in the lymphocytes at four hours, when they had decreased by 42 ± 8 per cent. These changes in the cells coincided with the change in the plasma iron level from a hyperferremic to a hypoferremic phase. At 24 hours when the level of iron was still depressed, the total leukocyte and polymorphonuclear counts were still elevated to 53 ± 19 and 55 ± 24 per cent above their initial levels. The drop in the level of eosinophils had decreased to 35 ± 13 per cent and the lymphocytes had returned to their pre-injection level or higher in all three dogs.

Following the intravenous injection in three dogs of 5 mg. per kg. of body weight of Dibenamine in 200 ml. of 0.9 per cent solution of sodium chloride, the changes noted in the cells were different from those obtained with the large doses. Two hours following the Dibenamine the mean per cent increase of the white blood cell and polymorphonuclear cell counts was 13 ± 13 and 32 ± 32. These increases were due entirely to increases in the total leukocyte and polymorphonuclear cell count of the one dog which developed a hypoferremia following this dose of Dibenamine. The eosinophils and lymphocytes had decreased at this period by 23 ± 11 and 37 ± 9 per cent. The in-

creases in the white blood cell and polymorphonuclear cell counts were maximal at six hours, being 34 ± 16 and 30 ± 11 per cent. At this period the eosinophils showed a maximal decrease of 41 ± 11 per cent. The maximal decrease of the lymphocytes occurred after four hours, at which time they had fallen to 29 ± 5 per cent below their initial level. All the changes noted in the cells were less than those obtained with the larger dose of Dibenamine although these differences were not statistically significant, due probably to the small number of dogs used.

The six dogs previously injected with Dibenamine were given a single intramuscular injection of 2 mg. of epinephrine. The changes in the white blood cell counts observed were very similar to those obtained in untreated dogs with epinephrine. Thus at six hours after the injection of the epinephrine there was a maximal increase in the total leukocyte count and polymorphonuclear cells of 53 ± 6 and 74 ± 11 per cent, respectively, and a maximal decrease of the eosinophils of 66 ± 5 per cent. The lymphocytes had fallen to their lowest value at four hours, the mean per cent decrease noted being 41 ± 6. Thus Dibenamine neither blocked the cellular changes nor the hypoferremia which followed the injection of epinephrine.

DISCUSSION

From these results it would appear that there is a relation between adrenocortical function and the level of plasma iron. Thus, it has been found that a variety of agents other than turpentine are capable of inducing hypoferremia in the dog. These include histamine, epinephrine, formaldehyde, fracture, anaphylactic shock and the mild stress of taking blood samples. The hypoferremia which followed epinephrine and histamine differed from that which followed turpentine in that maximal hypoferremia occurred in six to eight hours rather than in 24 to 48 hours.

Following the injection of adrenocortical extract and ACTH, hypoferremia developed at the same time as after histamine, epinephrine and anaphylactic shock, and to a similar degree. That the effect of ACTH was mediated through the adrenal cortex was demonstrated by the fact that in adrenalectomized dogs no hypoferremia was produced by the injection of ACTH. Hypoferremia caused by mild stress was also absent in the adrenalectomized dog. The hypoferremic effect of epinephrine was significantly less in adrenalectomized dogs than in intact animals ($P = < .001$) but epinephrine still produced a significant fall in plasma iron in the absence of the adrenal cortex ($P = < .01$).

Additional evidence of an association between the adrenal cortex and the level of plasma iron was afforded by the experiments on the rate of disappearance from the plasma of intravenously administered saccharated oxide of iron. This substance was removed most rapidly from the plasma of intact dogs given ACTH, and most slowly from the plasma of adrenalectomized dogs, the rate of disappearance in intact untreated animals falling between these two. The rate of disappearance from dogs previously given thorium dioxide was approximately equal to that of the adrenalectomized dogs. The urinary excretion of iron was not studied in these animals. Such studies are indicated and are now under way in this laboratory.

The correlation found in these studies between the production of lymphopenia with eosinopenia and hypoferremia may be adduced as further evidence of the association of the adrenal cortex with the mechanism of hypoferremia. Thus turpentine, epinephrine, and ACTH produced hypoferremia in intact dogs and they also produced a significant eosinopenia and lymphopenia. Following bleeding or the injection of saline, a significant fall in the eosinophils and lymphocytes only took place in those dogs which developed a hypoferremia. It has been shown by others that Dibenamine, in doses of 25 mg. per kg. of body weight, produces a fall in adrenal ascorbic acid concentration (15), and in this study the above dose of Dibenamine produced a hypoferremia which was accompanied by a significant eosinopenia and lymphopenia. Following the administration of 5 mg. per kg. of body weight of Dibenamine the one dog which developed hypoferremia at eight hours also showed significant cell changes. In the two dogs in which hypoferremia did not develop at eight hours the cell changes were much less pronounced. Furthermore in dogs pretreated with Dibenamine, epinephrine produced both hypoferremia and eosinopenia and lymphopenia.

In adrenalectomized animals turpentine was the

only substance which produced a significant mean per cent decrease in the circulating eosinophils, but even when turpentine was injected the decrease in the eosinophils in the adrenalectomized dogs was significantly less than the drop obtained in the intact animals ($P = < .01$). In the adrenalectomized dogs following the administration of ACTH the eosinophils showed a tendency to increase. This increase was significantly different from the decrease associated with hypoferremia produced in intact dogs ($P = < .001$). The hypoferremia produced by epinephrine in the intact dog was associated with a significant decrease in the circulating eosinophils. In the absence of the adrenal cortex, epinephrine produced a hypoferremia which was significantly less than that obtained in the intact dog and which was not associated with a significant decrease in the eosinophils. This is further evidence that the full hypoferremic effect of epinephrine can only be produced in the presence of the adrenal cortex, but it is also clear that it acts in part through another system.

If from the above evidence we assume that the adrenal cortex is one of the factors concerned in the regulation of the plasma iron level and specifically is concerned in the production of the hypoferremia of stress, the problem arises as to how this influence is mediated. In the previous paper in this series (3), it was demonstrated that the intravenous administration of colloidal thorium dioxide to dogs in amounts of 2 ml. per kg. of body weight per day for three consecutive days resulted in a marked hyperferremia and that under these circumstances the hypoferremia produced by the intramuscular injection of turpentine, even in doses as great as 5 ml., was almost entirely prevented. In the present investigation it has been shown that the hypoferremia producing effect of histamine is blocked in a similar manner. These findings together with the observation that iron accumulates in organs containing the cells of the reticulo-endothelial system under conditions of chronic stress (7, 16, 17) suggest that it is the reticulo-endothelial system which removes the iron from the plasma.

One possible explanation of the means whereby acute hypoferremia is produced following stress is that the cortical hormones stimulate the reticulo-endothelial system to take up the iron from the plasma. In support of this explanation is the observation of Gordon and Katsh (18) that in rats adrenalectomy results in a significant decrease in the uptake of thorium by the spleen while the administration of whole adrenal cortical extract significantly increases the accumulation of thorium in the spleens of adrenalectomized animals.

This explanation is admittedly entirely speculative at the present time. It is possible that ACTH and cortisone produce hypoferremia through an entirely different mechanism than does turpentine. Indeed, it has been shown in the present study that epinephrine and turpentine can produce some degree of hypoferremia even in adrenalectomized dogs. Whether or not this is by direct stimulation of the reticulo-endothelial system or through some intermediary channel other than the pituitary-adrenal axis, are possibilities which deserve consideration and are now under study in this laboratory.

The studies reported here in dogs have been repeated and extended in rats (19). In general, the results in rats have confirmed the dog studies.

It must be emphasized that the data presented in this paper concern the mechanism by which acute hypoferremia occurs in the dog. It is possible that the same mechanism is operative in the production of the chronic hypoferremia which accompanies chronic infections in man although no direct evidence in support of this has been presented in this paper. Indeed, to date we have not observed hypoferremia in man following the administration of ACTH. These studies are now being extended.

SUMMARY

1. It has been shown that a variety of agents other than bacterial and sterile turpentine abscesses produce acute hypoferremia in dogs. These include histamine, epinephrine, fracture, anaphylactic shock and mild stress.

2. The injection of adrenocortical extract and adrenocorticotrophic hormone produced a similar hypoferremia.

3. Adrenalectomy abolished the hypoferremia produced by mild stress and by ACTH; the hypoferremia-producing effect of epinephrine was significantly reduced in the adrenalectomized dog but was not abolished.

4. Intravenously administered saccharated oxide of iron disappeared rapidly from the plasma of intact dogs given ACTH and more slowly from the plasma of adrenalectomized dogs. The ad-

ministration of colloidal thorium dioxide also resulted in a decreased rate of disappearance of injected iron.

5. The intravenous injection of Dibenamine failed to block the hypoferremia-producing effect of epinephrine and itself produced a hyperferremia maximal in two hours and a hypoferremia which began in eight hours and was maximal at 24 hours.

6. When the various agents were employed, a correlation was observed between the degree of lymphopenia and eosinopenia produced and the degree of hypoferremia which developed.

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OBSERVATIONS DURING ACTH AND CORTISONE ADMINISTRATION TO A PATIENT WITH LONGSTANDING PANHYPOPITUITARISM AND RHEUMATOID ARTHRITIS [1]

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The opportunity to observe the effect of administration of pituitary adrenocorticotrophic hormone (ACTH) and 11-dehydro-17-hydroxycorticosterone (cortisone) on the moderately active rheumatoid arthritis of a patient with panhypopituitarism was presented us. The clinical features (1, 2) of hypopituitarism are in essence those of hypo-function of the so-called "target" organs of the pituitary; the adrenal cortex, thyroid, and gonads. Sheehan and Summers (1) report "rheumatism of joints particularly of the knees" in 13 of 93 cases of panhypopituitarism, but elucidate no further on this clinical feature. The physiologic effects of ACTH and cortisone are well known (3, 4), and the dramatic response of patients with rheumatoid arthritis to these agents has been amply recorded (3, 5, 6). Forsham, Thorn, Prunty, and Hills (7) noted an absence of normal initial response after administration of ACTH in the majority of cases with severe hypopituitarism, although in one case, full adrenal cortical activity was noted after six days of ACTH administration. Knowlton, Jailer, Hamilton, and West (8) reported minimal metabolic effects after administration of ACTH for 12 days in a patient with longstanding hypopituitarism. An increase in excretion of 17-ketosteroids and uric acid and evidence of sodium and chloride retention were the observed effects.

This study was undertaken in order to observe the following:

1. What direct action, if any, ACTH might have on the clinical and laboratory findings of rheumatoid arthritis, inasmuch as adrenal cortical function was apparently absent.

2. Whether return of adrenal cortical activity would become apparent after ACTH administration.

3. The response to cortisone.

Case Report: LER, a 46 year old white woman, was first seen in the out-patient clinic at University Hospitals in 1945, complaining of intermittent attacks of swelling and tenderness of the shoulders, wrists, fingers, knees, and ankles. Recent attacks were associated with fever, anorexia, and weight loss. A vague history of increased heat tolerance was obtained. The husband described episodes that suggested hypoglycemia which had occurred intermittently during the preceding seven years. Menstrual and obstetrical history revealed that the only pregnancy, 27 years previously, had terminated with a prolonged labor, associated with excessive blood loss. Lactation was minimal, and ceased entirely after seven days. Menstrual function was not present after the pregnancy. Examination showed an asthenic, poorly nourished woman with dry skin. There was diminution of axillary and pubic hair. Blood pressure was 100/70 mm. Hg. Minimal joint signs were evident. Basal metabolic rates were -22% and -27%. Erythrocyte sedimentation rate was 86 mm./hour (Westergren). Clinical diagnoses of hypothyroidism and rheumatoid arthritis were made. The patient was discharged on thyroid substance 0.1 gm. daily.

There were four subsequent clinic visits, the final one being in August, 1946. Thyroid substance had been used only sporadically. On each occasion the patient complained of tachycardia. Little change was observed in the general appearance. The erythrocyte sedimentation rate remained elevated. On one occasion, a typical rheumatoid lesion of a metacarpal-phalyngeal joint was noted.

She was admitted to the medical service in January, 1949. During the previous 30 months she had experienced progressive asthenia and increasing periods of sleep. Her only medication had been 30 mg. of thyroid substance daily. In recent months progressive swelling and tenderness in the right knee and left ankle had been observed. Examination showed a very torpid and listless woman whose weight was 106 pounds. The skin had no increased pigmentation and was smooth and dry. Axillary and pubic hair were sparse. The breasts were atrophic. The heart was small. Blood pressure was 70/50. Pelvic examination revealed an atrophic uterus and senile vaginitis. Fusiform swelling of slight degree was evident at the interphalyngeal joints of the hands. The knees were limited in range of motion and marked

[1] This study was supported by the Trust Fund of the Department of Internal Medicine which permitted purchase of ACTH from Armour & Co. and cortisone from Merck & Co., Inc.

crepitation on motion was noted. There was thickening of the periarticular tissues.

Shortly after admission, she had a febrile episode associated with a urinary tract infection and lapsed into complete unresponsiveness with further drop in blood pressure. Treatment for Addisonian crisis was instituted and resulted in a satisfactory response. An unsuccessful attempt to stabilize the blood pressure, electrolyte, and endocrine metabolism on desoxycorticosterone and thyroid extract was made. Eventual maintenance medications were: *1*) Lipoadrenal Cortex (Upjohn), *2* ml. intramuscularly daily. *2*) Testosterone propionate, 20 mg. intramuscularly daily. *3*) Sodium chloride, 6 gm. daily.

Laboratory examination at the time of admission failed to demonstrate the presence of urinary gonadotropins, and 17-ketosteroid excretion was 0.84 mg./24 hours. Serum sodium was 290 mg./100 ml., potassium 29.3 mg./100 ml., and plasma chlorides 525 mg./100 ml. Blood urea nitrogen was 17 mg./100 ml. and the creatinine 1.0 mg./100 ml. X-rays revealed the lung fields to be clear. The cardiothoracic ratio was 0.38. X-rays of the abdomen demonstrated no calcification in the adrenal areas. Skull x-rays were normal. Roentgenograms of the hands demonstrated atrophic changes. A basal metabolic rate two weeks after admission was −16%. A plasma protein bound iodine in June, 1950, was 2.6 gamma/100 ml. A test for adrenal cortical insufficiency with 25 mg. ACTH as described by Thorn, Forsham, Prunty, and Hills (9) failed to demonstrate a significant drop in circulating eosinophils or change in urinary uric acid excretion. An intravenous glucose tolerance test produced a febrile reaction and drop in blood pressure which necessitated discontinuance of the test and use of supplementary adrenal cortical extract and intravenous saline.

METHODS

During the study the carbohydrate, fat, protein, and sodium in the diet were calculated since it was impossible to keep the diet constant. Blood pressure and weight were recorded daily at 7:00 a.m. Because of the mental status of the patient 24 hour urine and stool specimens were collected with some difficulty. Toluene was used as preservative in the urine specimens except on days when 17-ketosteroid and uric acid determinations were made. Eosinophil counts and leukocyte counts were done by the method of Randolph (10); erythrocyte sedimentation rates by the Westergren method; serum so-

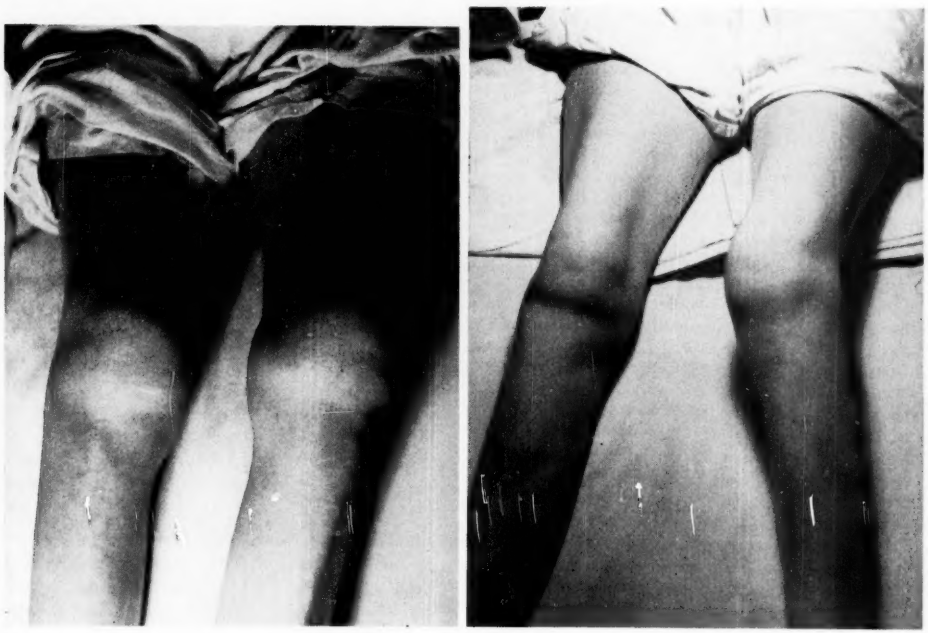

FIG. 1

A. PHOTOGRAPH OF THE KNEES TAKEN DURING THE INITIAL CONTROL PERIOD

B. PHOTOGRAPH AFTER THE SECOND COURSE OF CORTISONE, WITH DEFINITE DECREASE IN THE AMOUNT OF JOINT FLUID AND PERIARTICULAR SWELLING

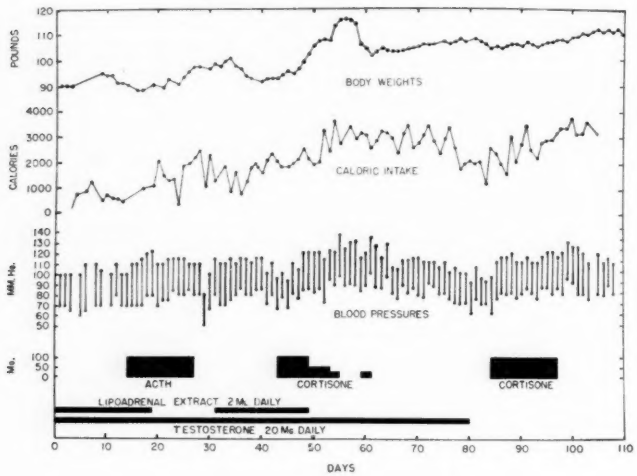

FIG. 2

dium by the method of Hoffman and Osgood (11); serum potassium according to Breh and Goebler (12); urine and blood uric acid by the method of Folin (13); 17-ketosteroids by the method of Robbie and Gibson (14). The patient was studied daily by two independent observers, and notations were made of changes in mental status, joint mobility, and general appearance. ACTH (Armour Standard LA-1-A Control J9801) freshly dissolved in saline was administered intramuscularly, 25 mg. every six hours. Cortisone acetate (Merck) was given every six hours intramuscularly (Figures 2, 3, 4).

Regimen (Figure 2): Function of the pituitary was thought to be so slight that to discontinue all medication at the start of ACTH administration was inadvisable. Lipoadrenal cortex was omitted after four days of ACTH, but testosterone was continued. Lipoadrenal cortex was again given at the termination of the course of ACTH when pneumonitis with fever and hypotension intervened. During the first period on cortisone, lipoadrenal cortex was discontinued when a sharp rise in weight and elevation of blood pressure occurred. Shortly thereafter cortisone was reduced and, later, discontinued when edema appeared. Added sodium chloride was also discontinued. Testosterone was discontinued before the second course of cortisone. At the completion of the second course of cortisone, no medications were given for 21 days. Fall in blood pressure necessitated resumption of therapy with lipoadrenal cortex.

RESULTS

Clinical observations: Before the beginning of this study, the patient was lethargic, passive and intermittently hallucinated. During the first six days of ACTH administration, hallucination and agitation become extreme, requiring restraint and on one occasion tube feeding. The psychotic behavior became less pronounced when ACTH was continued. While on cortisone the patient became cooperative, and had long periods of lucid behavior. During administration of cortisone the skin remained dry and soft. The subcutaneous tissue of the face took on a loose, spongy consistency much like that seen in myxedema. A luxuriant growth of axillary and pubic hair appeared during cortisone therapy. This was accompanied by extreme modesty, which heretofore had been lacking completely. The activity of the rheumatoid arthritis was such that no dramatic change was observed. During the course of ACTH there was slight objective increase in range of motion of the knees, ankles and left elbow. After four days on ACTH the patient walked spontaneously for the first time during the entire period of hospitalization. The agitation and hallucination at this period, however, probably accounted for the apparent absence of pain on walking. In the interval between ACTH and cortisone administration there was a definite exacerbation of joint pain, swelling and local heat. Cortisone brought about definite objective and subjective improvement in the rheumatoid state (Figure 1). This occurred gradually during a seven day period. During the 22 day period be-

tween the courses of cortisone there was no exacerbation of joint signs or symptoms. Twenty-one days after completion of the second course of cortisone, both knees but especially the right, became painful, warm, and swollen. An accompanying systemic febrile response occurred.

A gain in body weight of nine pounds was noted after 10 days of ACTH administration (Figure 2). No edema was evident. This gain could not be followed accurately because pneumonitis necessitated the use of supplementary adrenal cortical extract three days after completion of ACTH administration. By the sixth day of the first course of cortisone, a weight gain of 10 pounds had occurred. Edema appeared and remained despite discontinuation of lipoadrenal cortex and sodium chloride. Cortisone was discontinued on the 12th day after her weight had risen from 93 to 115 pounds. It was believed that the appearance of edema was a manifestation of sodium retaining effect of cortisone (3), added to the effect of testosterone (15). After edema had disappeared her weight remained 10 pounds above the control level and represented an increase in body tissue. This gain was maintained in the 23 day interval between courses of cortisone. During the second period of cortisone her weight increased but 3 pounds. However, an additional gain of 5 pounds was noted in the 12 day interval subsequent to its administration. During the second period, when cortisone was the only drug being administered, no edema appeared.

Blood pressure was maintained slightly above control levels by ACTH after discontinuation of

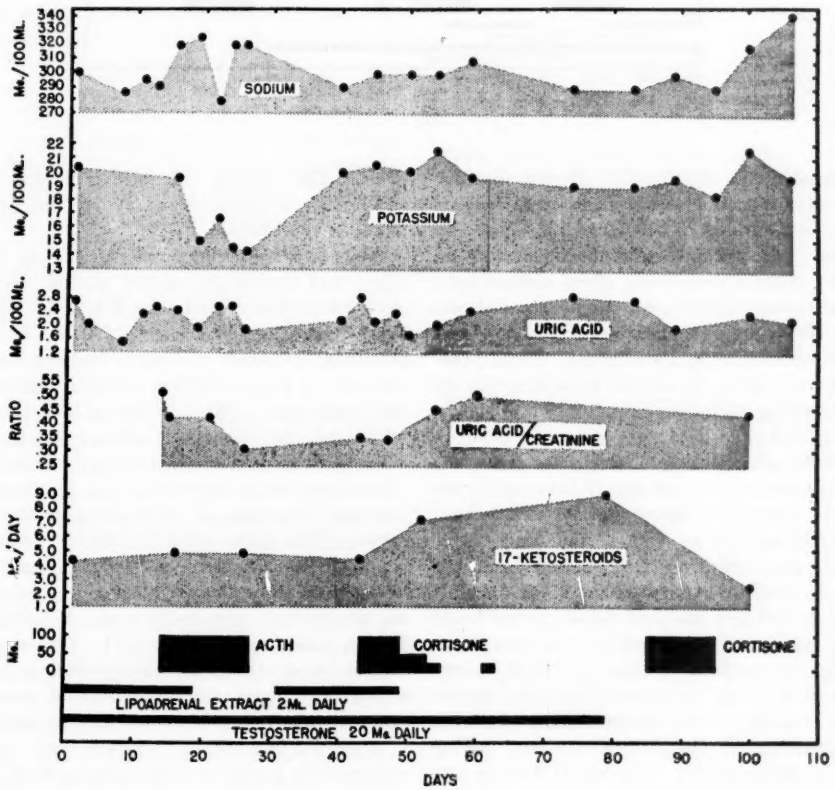

FIG. 3

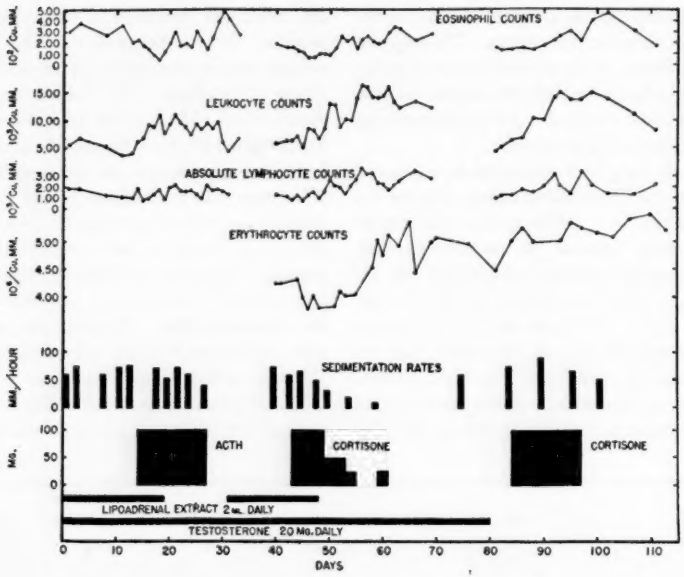

FIG. 4

lipoadrenal cortex (Figure 2). During the first course of cortisone, blood pressure rose from an average control level of 110/80 to 138/98 on the 12th day, concomitantly with the appearance of edema. When cortisone was given without lipoadrenal cortex, testosterone propionate and sodium chloride, only slight elevation of systolic and diastolic arterial tension was noted. Blood pressure did not return to control levels immediately after stopping cortisone, but dropped gradually over a period of 14–21 days.

Chemical observations: The data for serum sodium, potassium, uric acid, and 17-ketosteroids are given in Figure 3. Serum sodium varied from 280 to 335 mg./100 ml. with no significant correlation to drug administration. Serum potassium levels dropped to 14.9 mg./100 ml. on the fifth day of ACTH and remained below control levels during the entire course. This was interpreted as suggestive evidence of adrenal cortical activity inasmuch as potassium diuresis had been an observed effect of ACTH administration (3, 7, 8). No alteration of significance occurred during cortisone administration. Neither ACTH nor cortisone significantly altered the blood uric acid levels. The urinary uric acid/creatinine ratio was not significantly altered at any time in the course of this study.

While on testosterone propionate, 20 mg. daily, before and during the control period, 17-ketosteroid excretion ranged from 4.4 to 6.6 mg./24 hours. These figures are somewhat less than the percentage excretion of administered testosterone observed by Fraser, Forbes, Albright, Sulkowitch and Reifenstein (16). While ACTH was administered, no appreciable change in 17-ketosteroid excretion occurred. During the first course of cortisone, while testosterone was continued, an increased excretion of 17-ketosteroids was observed. This effect was noted 20 days after discontinuation of cortisone, and would suggest continued slow absorption from the site of injection, an observation previously made by Sprague, Power, Mason and Cluxton (17). Following the second course of cortisone during which no testosterone was given, the 17-ketosteroid excretion was maintained near the control level by cortisone.

Alteration in tolerance of carbohydrate was not evidenced by fasting and post-prandial blood sugar determinations.

Hematologic observations: Values for erythrocyte sedimentation rate, circulating eosinophils, total leukocytes, absolute lymphocytes, and erythrocytes are plotted in Figure 4. The erythrocyte sedimentation rate decreased from an average control value of 68 mm./hour at the termination of ACTH administration to 40 mm./hour. Significant decrease to 10 mm./hour occurred 16 days after initiation of cortisone therapy. During the second course of cortisone a significant decrease in sedimentation rates failed to occur. Total circulating eosinophils dropped from an average control level of 290/cu. mm. to 44/cu. mm. on the fourth day of ACTH therapy. A gradual return to essentially control level by the seventh day of treatment was noted. A similar decrease in circulating eosinophils was observed during the first course on cortisone, while no definite trend occurred during its second exhibition. The total leukocytes rose from an average control value of 5000/cu. mm. to average treatment values of 9000/cu. mm. on ACTH. A pronounced increase in total leukocyte count occurred after six days of cortisone and continued for eight days after its discontinuation. A similar increase was observed during the second course of cortisone. There was very little change in absolute lymphocyte counts during the entire study.

The erythrocyte count rose from control levels of 4,300,000/cu. mm. to 5,980,000/cu. mm. at the completion of the second course of cortisone.

DISCUSSION

Because this patient had clinical signs of hypothyroidism and a low basal metabolic rate, she was treated with small amounts of thyroid substance on four occasions. The response was adverse and each time the therapy had to be discontinued. The symptoms which caused the latest admission to the hospital suggested adrenal insufficiency, and although she was hypokinetic and her skin was dry the signs of myxedema were not outstanding. ACTH caused no alteration in the relationship of the clinical signs of adrenal insufficiency to those of hypothyroidism, but when the hypoadrenalism was controlled with cortisone, striking signs of myxedema occurred. Clinically it seemed that partial control of either adrenal or thyroid insufficiency enhanced signs of the untreated endocrine deficiency. That there is a relationship between adrenal and thyroid function has been demonstrated by Soffer, Gabrilove, and Jailer (18). Evidence that this relationship is more than relative is not apparent from our study.

The observations set forth in this study of an unusual clinical combination of rheumatoid arthritis and hypofunction of the adrenal cortex secondary to hypopituitarism are in keeping with the rapidly accumulating data in this field (3–6, 8). In our patient there was clinical and laboratory evidence that some bit of adrenal cortex remained and was responsive to stimulation by ACTH. This response mediated through the adrenal glands was shorter and less intense than the general response to substituted cortisone. Generalizations from this series of studies are not warranted.

SUMMARY

1. ACTH and cortisone were administered to a patient with longstanding panhypopituitarism and rheumatoid arthritis.

2. Partial adrenal cortical activity was demonstrated during ACTH administration.

3. The effect on the arthritis was equivocal after administration of ACTH, but was definite after administration of cortisone.

4. Clinical, chemical and hematologic data are in agreement with present conceptions of the metabolic and therapeutic properties of ACTH and cortisone.

ACKNOWLEDGMENTS

We wish to thank Mrs. Frances Jolly for the uric acid and creatinine determinations, Doctor R. B. Gibson for the sodium and potassium determinations and Miss Mary R. Bedford, dietician, for estimating the daily food consumption.

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THE ANEMIA OF THERMAL INJURY. I. STUDIES OF PIGMENT EXCRETION [1, 2]

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Our interest in the metabolism of hemoglobin in patients with thermal injury was stimulated by early observations that (*a*) in spite of multiple whole blood transfusions anemia often developed rapidly in the first 96 hours after injury, and (*b*) in the protracted convalescence of a patient with only a small burn, moderate anemia inevitably ensued. The latter was exceptionally refractory to all except replacement therapy. With these two facts in mind we have undertaken our present study of the burn patient in an attempt to answer the following questions: (*1*) to what extent does hemolysis occur, and (*2*) what information about the anemia of the protracted convalescent stage might a continuous investigation of heme metabolism give us?

Previous work on the same general problem has already been presented by Moore, Peacock, Blakeley, and Cope (1), who demonstrated an increased excretion of urobilinogen in the urine of four patients and an increased excretion in the feces in one patient. Similarly, the British workers, Anderson and Semeonoff (2), described increased excretion of urinary urobilinogen. Others have pointed out the potentiality of increased osmotic fragility and plasma hemolysis (3, 4) early in the post-burn period as well as agglutination and "sludging" of the red cells (5) in the peripheral circulation removed from the site of the injury.

Cartwright and his associates have presented several papers discussing some of the metabolic aspects of the anemia of chronic sepsis, and give data showing low serum iron, elevated serum copper and increased urinary coproporphyrin excretion (6). A few studies are available, notably those of Saifi and Vaughan (7, 8), in which estimations of the urobilinogen excretion in the urine and feces in chronic sepsis have been made. In our experience bacterial contamination and growth on the burned area is always present after the first dressing of third degree burns.

In the investigation of an anemia which appears suddenly over a period of a few days, it would seem of some importance to estimate the amount of the end products of hemoglobin catabolism. The correlation of the amount of hemoglobin end products, as urobilinogen, with the total number of grams of circulating hemoglobin has been discussed by Miller, Singer, and Dameshek (9) and others as the hemolytic index, and can serve as a rather reliable method of estimating intravascular hemolysis. Moore and his colleagues (1) in their review mention the initial hemolytic phase of burns but only support this observation with one case with fecal urobilinogen study. We believe the urine urobilinogen may have a different interpretation than that described.

METHODS AND MATERIAL

Seventeen burn patients admitted to the Burn Service of the Department of Surgery at the Medical College of Virginia were selected for study. The burns were usually of the free fire type caused by kerosene explosions in wood stoves and by blazing clothing. Immediately after admission the patients were started on study with an initial hemogram, a blood volume determination, and a continuous collection of all urine and feces. Subsequent blood studies were performed as indicated. Treatment in all the cases was essentially the same, employing pres-

[1] Presented in part before the Clinical Congress, American College of Surgeons, October 16–23, 1949, Chicago, Illinois.

[2] Supported by grants to the Surgical Research Laboratory, Department of Surgery, from the Medical Research and Development Board, Department of the Army (Project: S.F. No. W–49–007–MD–411).

[3] Markle Scholar in Medical Sciences; formerly, Postdoctorate Research Fellow of National Institutes of Health.

[4] Surgical Research Fellow.

[5] Director of Surgical Research Laboratory.

TABLE I

	Patient	Age	Sex	Race	Amount of burn in per cent		Cause	Result
					Third degree	Total		
Group I 0 to 10%	C. W.	17	M	W	0	35	Gasoline explosion	R*
	A. D.	13	F	W	1.5	23	Free fire	R
	R. G.	28	M	W	2.0	12	Electrical	R
	G. C.	10	M	W	4.0	70	Free fire	R
	J. B.	35	M	N	4.0	16.5	Hot water	R
	W. B.	11	M	N	7.0	11.0	Free fire	R
Group II 11% to 20%	B. C.	60	M	W	11.5	22.5	Free fire	R
	A. C.	19	M	N	12	17.5	Gasoline	R
	B. R.	15	M	N	15	20	Free fire	R
Group III 21% or greater	C. T.	31	M	N	30	47	Free fire	D*
	F. T.	33	M	N	30	95	Gasoline explosion	D
	J. M.	84	M	N	38	48	Free fire	D
	S. L.	57	F	W	20	70	Free fire	D
	G. L.	19	F	N	51	63	Free fire	D
	F. J.	20	F	N	55	65	Free fire	D
	O. B.	29	F	N	65	74.5	Free fire	D

* R = Recovered; D = Died.

sure bandage without debridement, parenteral antibiotics, fluids and when indicated whole blood transfusion.

The hematologic methods were those given by Wintrobe (10). Hemoglobin was measured by the alkaline hematin method in sodium carbonate on the Coleman Junior Spectrophotometer, standardized for hemoglobin by the oxygen capacity method. The urine was collected in brown glass bottles, using 5 Gm. of sodium carbonate with petroleum ether as a preservative. Feces were collected in waxed carton containers. Urine urobilinogen determinations were done on one day or two day collections, fecal urobilinogen on four day or longer collections by the quantitative method of Schwartz, Sborov, and Watson (11). We have used 200 mg. per day as the upper limit of normal for fecal urobilinogen studies since this was the highest value obtained on two normal subjects in our laboratory. This is somewhat lower than the upper limits usually described as 250 mg. per day. Total urinary coproporphyrin was determined on the Klett photofluorometer model 1040 by the method of Schwartz, Hawkinson, Cohen, and Watson (12). Standardization of our instrument was performed with coproporphyrin dimethyl ester, prepared, isolated, and purified in our laboratory from porphyria urine and urine from lead poisoned patients. Serum bilirubin (13), T-1824 plasma volume (14), and P^{32} red cell volume (15) were determined at intervals designed to aid us in our studies. The hemolytic index, a ratio of urobilinogen excretion per day to total circulating hemoglobin, has been described by Miller, Singer, and Dameshek (9). It is calculated in the following manner:

$$\text{hemolytic index} = \frac{\text{urobilinogen excretion in mg. per day} \times 100}{\text{total circulating hemoglobin in grams}}.$$

The normal range of this index is given as 11–21.

RESULTS AND DISCUSSION

Table I presents statistical data on our patients who were arbitrarily separated into three groups based on the percentage of third degree burn as estimated by the Lund and Browder values (16). This division is as follows: Group I, 0 to 10 per cent; Group II, 11 to 20 per cent; Group III, 21 per cent or greater.

The results of the fecal urobilinogen studies should be evaluated in the light of previous experience that whole blood transfusions, particularly if the blood has been stored for more than a week, leads in some instances, not only to a slight increase in the serum bilirubin, but also an increase in fecal urobilinogen (17, 18). Also of considerable importance are the recent clinical studies of London (19) and his associates which indicate that at least 11 per cent of the fecal stercobilin [6] in normal persons is derived from sources other than the pigment fraction of hemoglobin. To what extent increases in fecal urobilinogen (*vide infra*) are due to non-hemoglobin sources in our patients cannot be ascertained, and the possibility exists that tissue destruction is contributing tetrapyrrols, due to intracellular breakdown since all cells contain the ferroprotoporphyrin ring.

[6] The method used in this work measures both stercobilinogen and mesobilirubinogen and the result of the determination is expressed in terms of urobilinogen.

In Group I there were three instances in which fecal urobilinogen was considered to be increased as evaluated by the hemolytic index. In two of these the increase occurred in the first week and was associated with a marked drop in the hemoglobin and increased hemolytic index. We have interpreted this to mean excessive hemolysis. Neither of these patients received transfusions on admission. J. B. (Figure 1), 4 per cent third degree burns, illustrates an example of this response. Laboratory studies indicated a sharp rise in the fecal urobilinogen and a fall in the total circulating hemoglobin of 122 Gm. The hemolytic index increased from 3.8 to 37.4, later returning to within the accepted normal range of 11 to 21.

In a patient with 35 per cent second degree burn (C. W.) there was evidence of a definite hemolytic phase. It would appear, therefore, that hemolysis is not entirely a question of the depth of burn.

In two instances in Group I there is a different response. A. D. (Figure 2) with 1.5 per cent third degree burn, was given a total of 430 Gm. of hemoglobin shortly after admission raising her total circulating hemoglobin from 523 Gm. to 896 Gm., an increase of approximately 72 per cent. This gave rise to an "artificial" polycythemia. Interestingly enough, she did not show any excessive hemolysis as judged by the fecal urobilinogen studies. The hemolytic index of 10 was normal, but there was a moderate rise in the total serum bilirubin. The interpretation of this discrepancy is somewhat difficult. A possible explanation of this may be due to the difficulty in stool collection in this patient. It is noted that the fecal urobilinogen covers a period of eight days, whereas if the total amount of urobilinogen excreted during this period were computed on a three day basis, which it more nearly represents, the concentration of fecal urobilinogen in mg. per day would be correspondingly higher, and thus the hemolytic index. In addition as it has been pointed out by Ham and his associates (4), thermal

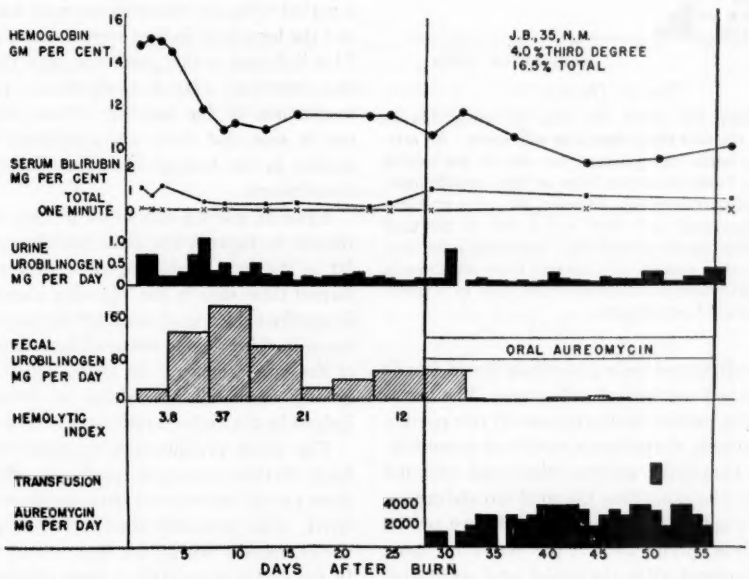

FIG. 1. (J. B.)

The graph demonstrates a drop in hemoglobin with a concomitant rise in the fecal urobilinogen and hemolytic index. Serum bilirubin and urine urobilinogen were unchanged. After the 28th day oral aureomycin was administered. Note the marked diminution in fecal urobilinogen output, without significant change in the urine urobilinogen. The hemolytic index after the 25th day was at the lower limit of normal and suggests decreased breakdown of heme pigments.

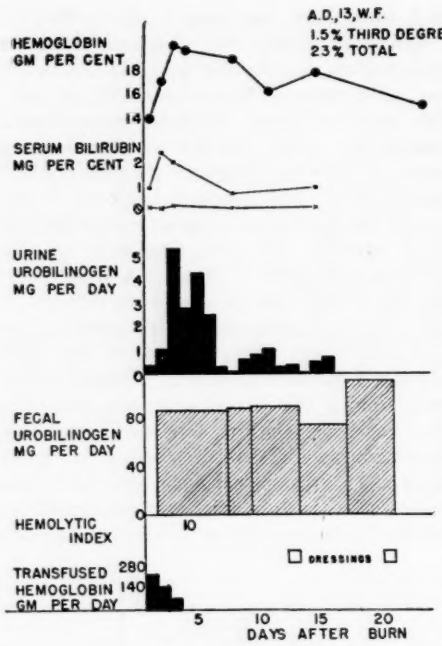

FIG. 2. (A. D.)

This patient was given 430 Gm. of hemoglobin as red cells in the first three days after admission. An artificial polycythemia was produced, but she did not exhibit any increase in the hemolytic index or fecal urobilinogen. On the third post burn day, however, the urine urobilinogen was increased, and there was a rise in the total serum bilirubin on the second day. Increased urine urobilinogen in the presence of a normal fecal urobilinogen suggests impairment of the ability of the liver to properly handle reabsorbed urobilinogen.

trauma leads to red cells potentially more fragile to osmotic and mechanical influences. The transfusion in this patient could give rise to two populations of red cells, the patient's, which are potentially subject to hemolysis, and the transfused cells, not fragile. At the same time the total circulating hemoglobin would have been raised. Hence on the basis of these speculations, if the hemolytic index were recalculated using the initial total circulating hemoglobin (prior to transfusion), the fecal urobilinogen on a three day basis, the index would have been 40. This of course fits well with the other findings of increased indirect serum bilirubin, and the data on the subsequent patients. The other patient who showed a different response was a youth of 10 years with a 4 per cent third degree burn. Although blood volume studies were not done, he did not show a significant rise in the fecal urobilinogen until the 10th day, when the fecal urobilinogen rose to 165 mg. per day.

In Group II are those with third degree burns covering from 11 to 20 per cent of the body surface. The fecal urobilinogen studies are altered by the use of oral or intravenous aureomycin, which will be discussed below.

In the severe group of burns, all of whom expired, there were marked increases in the fecal urobilinogen. Stools could not be collected in all instances but in those patients from whom specimens were obtained increases were all greater than 500 mg. per day, which is in the accepted range of the hemolytic anemias. In one instance, we were able to follow a man, F. T. (Figure 3), with 30 per cent third degree and 95 per cent total burn for a period of 33 days. At no time did his fecal urobilinogen return to normal even during a period when no transfusions were administered, and the hemolytic indices were constantly elevated. This indicates in this particular burn that continuous hemolysis played a significant part in the mechanism of the anemia. From the graph it can be seen that there was a constant and steady decline in the hemoglobin value despite multiple transfusions.

Table II gives a composite picture of the chief results as regards the fecal urobilinogen. In so far as the fecal urobilinogen is concerned, we interpret these data in the following manner. There is usually evidence of increased hemoglobin breakdown in third degree burns of less than 7 per cent of the body surface. In larger burns there is a greatly increased breakdown of hemoglobin as judged by the high excretions of fecal urobilinogen.

The urine urobilinogen appeared to follow a fairly distinct pattern in nearly all patients in the three groups unless oral aureomycin was administered. This consisted of an early rise to abnormal values, usually within the first two or three days. In the group of severely burned patients this substance attained very marked concentrations in the urine as shown in Table II. The urine urobilinogen values are tabulated as the highest one observed during hospitalization, and the value obtained on the third day after the burn. Group III

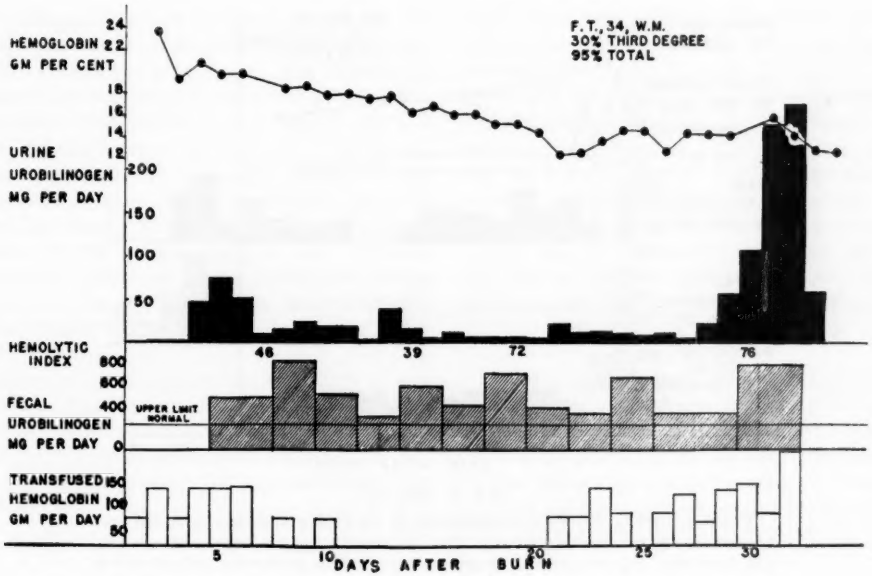

FIG. 3. (F. T.)

This patient with a severe burn demonstrated continuous and marked excretion of fecal urobilinogen and enormous increase in the urine urobilinogen immediately after injury and just before death. Despite daily transfusions except between the 11th and 20th days his hemoglobin dropped steadily, likewise during this same period the fecal urobilinogen remained elevated, and the hemolytic index was continuously increased.

TABLE II

Urine and fecal urobilinogen, hemolytic index, and serum bilirubin

	Patient	Amount of burn		Urine urobilinogen (*mg. per day*)		Fecal urobilinogen		Serum bilirubin		Hemolytic index	Aureomycin
		Third degree	Total	Highest	Third day post burn	(*mg. per day*)	Post burn day	1′	Total		
Group I	C. W.	0	35	18	18	300	6	0.12	2.4	34.2	No
	A. D.	1.5	33	5.4	5.4	90	6	0.2	2.4	10.1	No
	R. G.	3.0	12	32.0	11.0	215	6	—	—	—	No
	G. C.	4.0	70	8.2	1.5	90	4	0.2	0.8	—	No
	J. B.	4.0	16.5	1.2	0.3	180	8	0.1	1.1	37.4	No
	W. B.	7.0	7.0	20	3.4	150	4	0.4	0.85	49.7	No
Group II	A. C.	11.5	22.5	42	37.0	—*	—	—	—	—	No
	B. C.	12.0	17.5	0.2	0.1	0.6	2	0.32	4.06	—	Yes
	B. R.	15.0	20	6.0	6.0	10.6	4	0.16	0.23	—	Yes (I.V.)
Group III	C. T.	30	42	250	250	530	5	0.2	2.3	—	No
	F. T.	30	95	275	45	800	5	0.6	1.2	37	No
	J. M.	38	48	157	6.0	582	11	0.4	1.9	54	No
	S. L.	?	70	67	11	—§	—	1.34	2.17	—	No
	G. L.	51	63	32	14	23†	4	4.6	7.55	—	No
	F. J.	55	65	0.15	0.1	6.3‡	12	—	—	—	Yes
	O. B.	65	75	0.8	0.7‡	—	3	1.7	5.39	—	Yes

* Diarrhea incomplete collection.
† Only 40 gm. of feces obtained.
‡ No feces first nine days.
§ Feces lost.

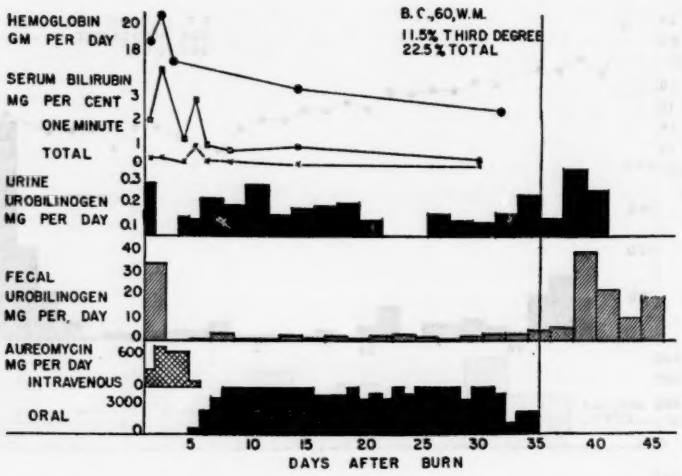

FIG. 4. (B. C.)

This demonstrates the effect of aureomycin on the fecal urobilinogen from the time of admission to the 35th hospital day. Oral aureomycin was not begun until the fifth post burn day, and the effect of intravenous aureomycin during the early period is equivocal. Note the drop in the hemoglobin and the rise in the serum bilirubin. Hemolytic indices could not be run because of the aureomycin effect.

patients show the most marked concentrations, but elevations are present in all except those receiving aureomycin and in one patient with uremia and almost complete anuria. Both Figures 2 and 3, are representative examples of the changes seen in our patients. This increased excretion of urobilinogen in the urine we interpret as evidence of early and rather marked hepatic failure, failure to properly catabolize the portal blood urobilinogen. Hence it appears in the peripheral blood and is excreted in the urine. This subject is to be dealt with more fully in a later paper.

One patient in Group I, J. B. (Figure 1); two patients in Group II, B. R. and B. C. (Figure 4); and two patients in the severely burned group, F. J. and O. B., were given either oral or parenteral aureomycin, or both, to observe the effect of this antibiotic on the excretion of urobilinogen in the urine and stool.

Briefly, bilirubin derived from hemoglobin is reduced by bacteria in the gastro-intestinal tract to mesobilirubinogen and stercobilinogen, both colorless compounds and conveniently grouped together with the name of urobilinogen because of an identical chromogenic reaction with Ehrlich's reagent. These are apparently entirely of enterogenous origin. Reabsorption of the urobilinogen takes place in the gut, and the liver effectively removes or catabolizes these substances in such a manner that only a small amount, up to 3 mg. per day (actually in our experience only 1.5 mg. per day), is excreted in the urine.

Sborov and his associates have already discovered the effects of aureomycin on fecal urobilinogen due to its sterilizing effect on the fecal flora (20). Our experience in finding a marked reduction is nearly the same as theirs. It is interesting to record, however, that petroleum ether-soluble Ehrlich-reacting substances, presumably tetrapyrroles, were still present. These effects with aureomycin [7] are shown graphically in Figures 1 and 4.

B. R. (Table II) who suffered about 15 per cent third degree burn received both intravenous and oral aureomycin from the onset but severe nausea and vomiting make it uncertain as to how much oral aureomycin was retained. We observed that

[7] The aureomycin for these studies was generously supplied by the Lederle Laboratories Division, American Cyanamid Company.

no increase of the fecal urobilinogen occurred, but a significant rise in urine urobilinogen was evident. After aureomycin was stopped, and following a transfusion there was a sharp rise in fecal urobilinogen. The patient B. C. (Table II) with 12 per cent third degree burn, received intravenous aureomycin initially with equivocal effect on the fecal urobilinogen. After the administration of oral aureomycin the amount of Ehrlich-reacting substances was less than 5 mg. per day. Likewise the urine urobilinogen was exceedingly low giving values of 0.1 to 0.2 mg. per day. Four days after discontinuing aureomycin the fecal urobilinogen rose to values which we have observed commonly in the convalescent phase of the moderately burned patient. The fecal urobilinogen values were extremely low in a previous patient discussed (J. B., Figure 1), ranging between 0.6 and 6 mg. per day during the time when he was receiving oral aureomycin.

As shown previously there is, in the severe group, a marked increase in the fecal urobilinogen, but in one patient, F. J., with 55 per cent third degree burn who received aureomycin, and in whom other evidence of hemolysis was present, we observed the fecal urobilinogen did not increase, and the amount of Ehrlich-reacting compounds remained essentially the same, *i.e.*, less than 5 mg. per day and usually about 1.2 mg. per day. Likewise, the urine urobilinogen in this patient was extremely low ranging from 0.1 to 0.2 mg. per day. These observations naturally raise the question of whether the Ehrlich-reacting substance present after aureomycin is a urobilinogen, since it is rather constant even when the total amount of petroleum ether-soluble Ehrlich-reacting compounds varies over a wide range.

The total urinary coproporphyrin excretion was studied in six patients who are in the following categories. Two in Group I, three in Group II, and one in Group III. Since this determination was not begun until late in the study, it does not include all patients. There was an increased excretion of total urinary coproporphyrin in all patients studied, and it did not appear to vary with severity of the burn. In four of these, normal

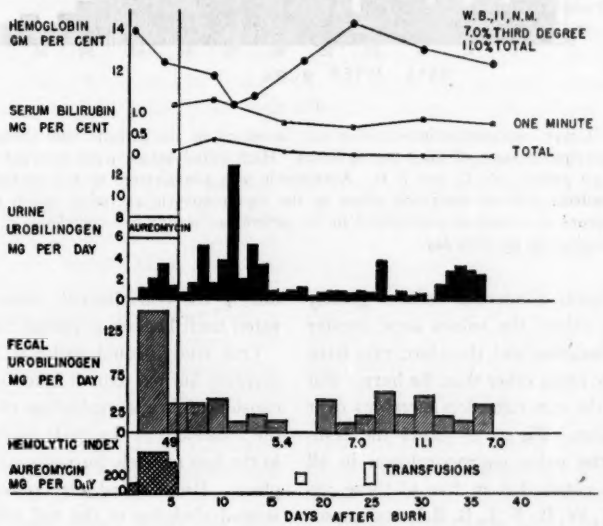

FIG. 5. (W. B.)

In this patient fecal urobilinogen was initially increased and intravenous aureomycin had no effect on either the fecal or urine urobilinogen excretion. Note the increased hemolytic index with a concomitant drop in hemoglobin. After two weeks and during convalescence the hemolytic index was low suggesting decreased heme breakdown.

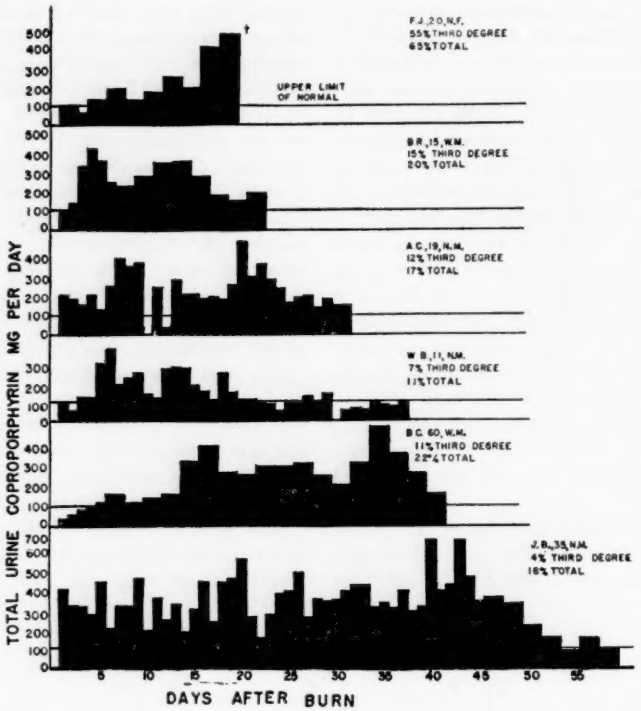

FIG. 6.

Urine coproporphyrin excretion was increased in six patients who showed varying amounts of third degree burns. High initial values were observed in two patients, A. C. and J. B. Aureomycin was administered to five of these patients with no observable effect on the coproporphyrin excretion, which returned to normal or near normal in five patients as healing was complete. F. J. expired on the 19th day.

values obtained upon admission became greatly elevated. In two others the values were greater than normal on admission and, therefore, may have resulted from some cause other than the burn. But in these patients the concentration increases over the admission value. Figure 6 shows the composite graph of the urine coproporphyrin in all patients. It was noted that in five of these patients (J. B., I. C., W. B., F. J., B. R.), oral aureomycin had no effect on the coproporphyrin excretion. Isomer analysis was not performed on any of these urines so that distribution of types cannot be determined. It would appear that the excretion of urinary coproporphyrin is the greatest during the convalescent phase and remains elevated until healing is almost complete.

Our study would make it appear that in the severely burned patient hemolysis must always be considered in the evaluation of the anemia and in the treatment of the early postburn phase. Even in the less severely burned patient hemolysis takes place. Brooks and his colleagues have demonstrated sludging of the red cells with subsequent agglutination and phagocytosis (21). Red cell destruction is associated with the opening of the porphyrin ring and subsequent degradation of hemoglobin to open chain tetrapyrroles. In our patients the drop in hemoglobin, the rise in hemolytic

index, and often a rise in the indirect type of serum bilirubin are other experimental data to support this concept. In one severely burned patient there was evidence of hemolysis from admission to demise 32 days later.

In the study of the burn patient during convalescence and grafting, the ratio of the fecal urobilinogen to the total circulating hemoglobin would seem to point to decreased hemolytic activity, which may result from decreased hemoglobin synthesis. Actually the hemolytic index is lowered (W. B., Figure 5). Increased coproporphyrin excretion may also be a reflection of a disturbance in hemoglobin synthesis.

The synthesis component of the anemia of burns has been very thoroughly discussed by Moore and his associates (1) and they have used the method of estimating the uptake of radioactive iron as a guide in evaluating the marrow depression. One study showing a low fecal urobilinogen excretion, and hence hemoglobin catabolism, with low hemolytic indices in the convalescent phase, suggests a decreased breakdown (Figure 5). We believe that one of the most important factors in the apparent marrow depression is the presence of bacterial growth on the burned area. The urinary coproporphyrin studies during the convalescent phase may favor this idea. However, the urinary coproporphyrin findings may be evidence of liver malfunction.

Moore and his colleagues (1) described increases in urine urobilinogen in their patients, and our data is in agreement with their work. We interpret differently the increased quantity of urine urobilinogen even though there is an increased amount of fecal urobilinogen in the stool. The level of the urinary urobilinogen is probably a very sensitive index of liver cell function. In burns, what we are measuring by the urobilinogen excretion is not only evidence of hemolysis but also impairment of the ability of the liver to properly catabolize the increased amounts of this substance.

The present studies would seem to answer the question concerning the occurrence of hemolysis. We believe that hemolysis occurs extensively, particularly in the large burn, and must always be reckoned with in evaluating these patients. This study has not answered the second question entirely to our satisfaction. A continuous investigation of heme catabolism shows us there is a decreased breakdown of heme products during the convalescent phase of burns. It gives us very little insight into the mechanisms involved in this process. In severely burned patients, during the long convalescent phase there may be considerable hemolysis taking place as evidenced by an increased hemolytic index and an increased fecal urobilinogen. Increased breakdown coupled with a depressed bone marrow would make the anemia more severe. As our work has indicated in one patient, in spite of a repeated transfusion, anemia progressed rapidly.

No conclusion can be drawn about the effect of aureomycin as far as anemia of thermal burns is concerned, but we might draw some interesting speculation about urobilinogen metabolism and Ehrlich-reacting substances in the feces in patients who are receiving oral aureomycin. It would appear that aureomycin can effectively sterilize the gastro-intestinal tract, so that at least the main reduction agents of bilirubin are inhibited to such an extent that little or no reduction of bilirubin occurs. We, nevertheless, consistently found in the feces a small amount of Ehrlich-reacting substances. Whether these small quantities are separate and entirely different compounds from urobilinogen we are unable to say. We are tempted to think they may be other substances since, in patients in whom we expect large increases of urobilinogen, the detectible quantity of these compounds remains almost the same as that in patients who are excreting only an expected small amount of urobilinogen.

CONCLUSIONS

1. Hemolysis, as judged by fecal urobilinogen excretion compared to total circulating hemoglobin, occurs in burns of all degrees. It is very great in third degree burns of more than 20 per cent.

2. There is usually a constant increase in the urinary urobilinogen noted on the third day after injury and this probably denotes early hepatic dysfunction and inability to properly catabolize an increased amount of urobilinogen.

3. Oral aureomycin effectively reduces the fecal and urinary urobilinogen to very small amounts. It is questioned whether the petroleum ether-soluble Ehrlich-reacting substances found after oral aureomycin administration are urobilinogens or whether they are entirely different compounds.

4. Additional evidence is presented to show that the anemia of thermal burns is at first hemolytic, then dyshemopoietic.

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THE ANEMIA OF THERMAL INJURY. II. STUDIES OF LIVER FUNCTION [1, 2]

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In a previous communication it was demonstrated that an increased excretion of urine urobilinogen was an early and constant finding in thermal injury even in burns of a relatively small amount of third degree (1). Since this particular finding was interpreted by us as a manifestation of hepatic injury, we undertook the further study of certain liver function tests in burned patients, none of whom received sulfonamides, tannic acid or other escharing therapy.

Bardeen published a review of the pathology of severe burns in 1899 and noted at that time changes in the liver and the kidney (2). Wolff, Elkinton and Rhoads demonstrated in three burn patients B.S.P. retention, slight icterus, and a decreased glucose tolerance as well as a decreased hippuric acid excretion test for liver function (3), and Boyce records similar observations and remarks upon the manifestation of jaundice in 65 per cent of the burn patients reported by him (4).

The histopathology of liver changes from 21 to 57 hours after injury has been described as fatty metamorphosis of the epithelial cells in the central zone of the hepatic lobules, and nuclear damage, especially karyolysis. After 57 hours there is slight enlargement, the liver is lighter yellow, greasy, friable, and has venous congestion of the "nutmeg" type. In addition there is evidence of degeneration and necrosis (5). It was believed that the central rather than the peripheral zone was first affected. Gillman and Gillman examined the livers of 38 patients dying within one hour and up to 36 days after burns and showed fatty changes and atrophy of the cells in the central zone. Eight days seemed to be necessary for the development of a very fatty liver, and it would appear that tannic acid intensified the tendency toward necrosis, which they believe present in some patients with burns. However, the majority of the microscopic liver changes were insufficient to account for a fatal termination. They concluded that if the liver contributed to death it did so through altered biochemical effects. It was the opinion of some that tannic acid therapy was responsible for precipitating liver damage (6) and the liver damage from tannic acid was central in character (7). Duffin reports an autopsied case with severe liver failure (8). Hepatic necrosis by his standard occurred in only six patients. Baker made an analysis of 87 autopsied burns on whom liver sections were available. In 56 death occurred before the sixth day. In only three of these was necrosis sufficient to involve nearly one-half the liver lobule, and all of these succumbed on the eighth day after burning. None received tannic acid. He concludes that minimal hepatic necrosis may apparently be caused by burns. Histologically demonstrable fat was frequently seen in those patients dying in the first 24 hours, and in those dying after four weeks. He concludes that necrosis of the liver is not a lesion characteristic of burns (9).

Hartmann and Romence studied liver damage in dogs and found gross enlargement of both the liver and spleen (10). In acute experiments of four to ten days, histopathology demonstrated hepatic lesions ranging from marked congestion of the sinusoids and compression of the liver cords

[1] Presented in part before the Clinical Congress, American College of Surgeons, October 16–23, 1949, Chicago, Illinois.

[2] Supported by grants to the Surgical Research Laboratory, Department of Surgery, from the Medical Research and Development Board, Department of the Army (Project: S.F. No. W–49–005–MD–411).

[3] Markle Scholar in Medical Sciences, formerly Post doctorate Research Fellow of the National Institutes of Health.

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to varying amounts of granular, vascular, and fatty degeneration. Central necrosis occurred in eight to ten days, and liver function was depressed. They did not find the picture of acute liver insufficiency. Belt (11) believes the liver necrosis following burns simulates the lesions of yellow fever and described Councilman bodies and nuclear inclusion bodies. His patients had tannic acid treatment.

METHODS AND MATERIALS

Fifteen burn patients admitted to the Burn Service of the Department of Surgery of the Medical College of Virginia were selected for study. The majority of the burns were usually of the free fire type from kerosene explosions on wood stoves or blazing clothing. Blood for liver function tests was taken on admission and repeated at intervals during the course of the treatment. In some instances only one set of determinations could be obtained because of early demise or difficulty in obtaining blood. Treatment in all cases was essentially the same and employing pressure bandages without debridement. Parenteral antibiotics, whole blood and replacement fluid therapy were used as needed clinically. No tannic acid, escharing agent, or sulfonamides were employed. In some instances the patients during the course of study were on a special liquid diet providing 100 Gm. of protein, with sufficient fat and carbohydrate to give an average of 45 Calories per kilogram, and vitamin supplements as recommended by the National Research Council.

The following liver function tests were performed: Bromsulfalein retention using 5 mg. per kilogram and taking a 45 minute sample (12); cephalin-cholesterol flocculation at the modified pH of 7.4 (13); thymol turbidity (14); prothrombin time employing the one stage method of Quick (15); total protein and albumin-globulin ratio (16); the serum bilirubin employing the Ducci-Watson modification of the Malloy and Evelyn technique (17); and the quantitative urine urobilinogen (18).

As in a previous study the patients were separated into three clinical groups on the basis of the amount of surface area involved by third degree burn. There were five patients in Group I who had burns of from 0 to 11 per cent third degree; three in Group II, 12–20 per cent; and seven whose burns were greater than 21 per cent in Group III.

Autopsy material was obtained on five of the six patients in Group III, all of whom expired.

RESULTS AND DISCUSSION

Table I gives the composite results of the liver function tests on all 15 patients as performed within the first ten days (usually within the first five days) after the burn. The most constant finding is an increased amount of urobilinogen in the urine, which in one instance reached as high as 75 mg. per day.

In the five patients who had less than 10 per cent third degree burns we see there was bromsulfalein retention of greater than 20 per cent in two of the three patients in whom this test was performed. In four of the five patients there was an elevation of the thymol turbidity test to greater than four units which is considered the upper limit of normal in our laboratory. Two of the four patients in whom the cephalin-cholesterol floccula-

TABLE I

Composite table of liver function studies

	Patient	Age	Sex	B.S.P. retention	Cephalin flocculation	Thymol turbidity	Prothrombin concentration	Urine urobilinogen	Total protein	Albumin	Globulin	Albumin-globulin ratio	Serum bilirubin	
													1 min.	total
				per cent		*units*	*per cent*	*mg. per day*	*Gm. per cent*	*Gm. per cent*	*Gm. per cent*		*mg. per cent*	*mg. per cent*
Group I	J. B.	35	M	21	3	7	75	1.2	5.8	2.75	3.09	0.87	0.02	0.40
	C. W.	17	M	2.7	1	6	43	17.0	5.9	3.7	2.2	1.68	0.24	2.52
	W. B.	11	M	32	1	7	60	5.2	6.95	3.1	3.85	0.80	0.4	0.85
	G. C.	10	M	—	—	4	—	3.8	4.8	2.6	2.2	1.18	0.25	0.45
	A. D.	13	F	—	4	2	—	6.1	6.4	3.1	3.3	0.94	0.07	2.45
Group II	B. C.	60	M	23	3	5	19	0.1	6.0	3.4	3.6	0.90	0.32	4.06
	B. R.	15	M	7.9	3	3	60	6.0	6.15	2.84	3.21	0.88	0.16	0.23
	A. C.	19	M	19	0	6	43	40.0	7.25	4.0	3.25	1.23	—	—
Group III	O. B.	29	F	48	0	15	43	0.18	5.8	2.3	3.5	0.66	1.7	5.39
	G. L.	19	F	18	1	2	—	32	5.9	3.3	2.6	1.27	4.6	7.55
	S. L.	57	F	28	3	4	75	65	6.7	2.8	3.9	0.71	1.34	2.17
	F. J.	20	F	31	3	2	33	—	4.8	2.05	2.75	0.74	—	—
	C. T.	31	M	52.8	4	1	—	250	5.5	3.1	2.4	1.29	9.02	13.85
	F. T.	31	M	19.0	4	—	—	274	4.3	2.4	1.9	1.26	0.7	1.2

TABLE II

J. B., N. M., 35, 4 per cent third degree burn

Date	Days post-burn	B.S.P. retention	Cephalin flocculation	Thymol turbidity	Prothrombin concentration	Total protein	Albumin	Globulin	Serum bilirubin		A/G ratio
									1 min.	total	
		per cent		*units*	*per cent*	*Gm. per cent*	*Gm. per cent*	*Gm. per cent*	*mg. per cent*	*mg. per cent*	
10–26	1	12		1	100	6.58	3.87	2.71	0.12	1.09	1.43
10–27	2					5.37	3.10	2.17	0.07	0.71	1.43
10–28	3				75	5.58	2.75	2.83			0.97
11–1	7	14.4		5	100	5.8	2.71	3.09	0.11	0.40	0.87
11–3	9								0.11	0.18	
11–4	10	21.3	3+	7	75	6.14	3.35	2.79			1.20
11–7	13								0.02	0.30	
11–10	16								0.01	0.46	
11–12	18	19		1	75	6.10	2.58	3.52			0.73
11–17	23								0.02	0.12	
11–19	25			2	50	6.50	3.6	2.1	0.07	0.46	1.16
11–23	29	5.4							0.05	1.03	
11–28	35			2.5	60	6.24	3.1	3.14			0.98
11–30	37								0.18	0.55	
12–1	38	22.8							0.14	0.56	
12–3	40			2	75	5.6	2.84	2.76			1.03
12–8	45	25.0							0.15	0.71	
12–10	47			2	60	5.6	2.6	3.0			0.86
12–15	52								0.10	0.28	
12–16	53	10.9									
12–17	54			2	38	6.20	3.35	2.85			1.17
12–22	59	4.6		3	60	5.8	2.84	2.96	0.11	0.56	1.15

tion tests were done showed elevated values. Total serum proteins were slightly lowered in all except one of these patients, and the albumin-globulin ratios were decreased.

In one patient who was followed for a period of about 60 days, and whose protocol is shown in Table II we see a constant bromsulfalein retention until the time of complete healing. Cephalin flocculation, and thymol turbidity were increased at first, but the thymol turbidity was not abnormal after the tenth post-burn day. In this instance repeated determination of the serum bilirubin was normal, and the urine urobilinogen excretion did not become abnormal at any time, but the urinary coproporphyrin [6] was definitely elevated until just before complete healing was accomplished and the patient discharged. The total protein data showed an initial low normal total protein with a normal ratio of albumin and globulin, but by the seventh day the albumin fraction had decreased so that the globulin was now greater, and there remained a disturbance of this ratio for the next 40 days, despite an adequate protein intake of over 100 Gm. per day with a caloric intake of 45 Calories per kilogram. Identical changes in the total protein and albumin-globulin ratios were observed in the three other patients in this group. The only patient who did not show the reversal of the A/G ratio and no decrease in the total protein was a boy with a 35 per cent first and second degree burn. He also did not show any change in the B.S.P. retention.

In three patients (Table I) who had greater than 10 per cent third degree burn, but less than 20 per cent burn, all showed B.S.P. retention, but in one of them it was only 7.9 per cent. The cephalin flocculation was abnormal in two patients but the changes in the thymol turbidity were not marked. Two patients received aureomycin and in one the urine urobilinogen excretion was modified by this drug. The other, B. R., had an increased excretion of urinary urobilinogen probably because severe nausea and vomiting prevented retention of the oral aureomycin. Table I gives the other data collected for these three patients.

In one patient, B. C., who was followed for as long as 135 days there was an abnormal B.S.P. retention for over a month. Table III gives the complete data. He had an increased "indirect" serum bilirubin the second day after the burn. In spite of an adequate diet with a protein intake of over 100 Gm. daily there was a marked alteration of the albumin-globulin ratio and a lowered total se-

[6] These data have been presented elsewhere (1).

TABLE III

B. C., W. M., 60, 11.5 per cent third degree burn

Date	Days post-burn	B.S.P. retention	Cephalin flocculation	Thymol turbidity	Prothrombin concentration	Total protein	Albumin	Globulin	A/G ratio	Serum bilirubin	
										1min.	total
		per cent		*units*	*per cent*	*Gm. per cent*	*Gm. per cent*	*Gm. per cent*		*mg. per cent*	*mg. per cent*
12–9–49	0			5	75	6.1	4.4	1.7	2.58	0.3	1.95
12–10	1	22.3	0		60	6.0	3.4	3.6	0.9	0.32	4.06
12–12	3		3+	2	50					0.91	2.89
12–15	6	23.1				4.8	3.1	1.7	1.82		
12–16	7									0.12	0.65
12–17	8		0	3	27	5.2	2.84	2.36	1.2		
12–20	11				100						
12–22	13	13.2	0	3	60	4.6	2.3	2.3	1.0	0.08	0.80
12–27	18		0	2	50	5.0	1.6	3.4	0.47		
12–30	21	12.2									
1–4–50	26				100	5.6	1.7	3.95	0.43		
1–7	29	2.79								0.05	0.22
1–9	31			4.7							
1–10	32		0								
1–30	52		0	9.0	60	6.63	2.85	3.78	0.75		
2–9	62		0	9.5	100	5.4	2.6	2.8	0.93		
2–21	74					6.45	3.2	3.2	1.0		
3–15	96		0	7.0		6.39	2.5	3.89	0.64		
3–28	109					5.73	3.0	2.73	1.10		
4–7	119		2+	8.0	100	6.18	3.3	2.8	1.18		
4–19	130					6.97					
4–24	135					5.36	3.58	1.78	2.05		

rum protein. Even at 96 days following the burn total serum proteins were still abnormal. The same changes were observed in two other patients, both of whom had adequate dietary intake.

In six patients who had burns of greater than 20 per cent third degree the changes in the liver function were seemingly much more marked. All patients had a marked retention of the B.S.P. dye. In all except two the cephalin flocculation was three plus or greater and there was a marked increase in the urine urobilinogen in all except one (O. B.) who developed severe oliguria and uremia. Four patients developed jaundice and five showed significant increases in the one minute fraction of the bilirubin determination. One man in this group was followed for a period of 32 days before his death and he developed outstanding changes in the total protein and albumin-globulin ratio. The albumin fraction remained for long periods around 1 Gm. and the total protein slightly over 5 Gm. per cent. These data are presented in Table IV. Electrophoretic studies of the serum proteins were performed on many of the patients reported in this paper. Changes were evident in all the protein fractions. This work is to be reported elsewhere (19).

In all third degree burns from the small to the most extensive there appears to be involvement of the functional state of the liver as judged by the usual liver function tests. It is suggested by our data that the more extensive the burn the greater the functional damage.

All of our patients who had more than the 20 per cent third degree burn succumbed and autopsies were performed on five of the six. The autopsy material demonstrated pathologic changes in the

TABLE IV

F. T., W. M., 32, 30 per cent third degree burn

Date	Days post burn	Total protein	Albumin	Globulin	A/G ratio
		Gm. per cent	*Gm. per cent*	*Gm. per cent*	
9–22	0				
9–23	1	4.8	3.2	1.6	2.0
9–24	2	4.7	2.9	1.8	1.61
9–25	3	3.6			
9–26	4	5.0			
9–27	5	4.3	2.4	1.9	1.26
1–4	12	5.1	1.3	3.8	0.33
1–7	15	5.1	1.1	4.0	0.37
1–11	19	5.2	1.0	4.5	0.22
1–14	21	5.5	1.7	3.8	0.44
1–15	22	4.9	1.7	3.8	0.44
1–18	25	6.2	2.9	3.3	0.88
1–28	35	4.8	2.4	2.4	1.00

livers of all the patients who died. There did not appear to be any definite pattern of pathologic alteration. In one man, F. T., with 30 per cent third degree and 95 per cent total burn who lived for 32 days the histology of the liver showed central vein changes, with sinusoidal congestion and generalized cloudy swelling and fat deposition more marked in the peripheral zones of the liver lobule. Many of the von Kupfer cells contained yellowish pigment, slightly granular in quality, which might be iron. These changes may be seen in Figures 1 and 1a. In another, O. B., a 29 year old negro female with 65 per cent third degree, there was a marked central congestion, degeneration, and necrosis, with an accumulation of polymorphonuclear neutrophiles in this area. As in F. T., yellowish brown pigment was prominent in the von Kupfer cells. In the areas of necrosis were observed macrophages with eosinophilic cytoplasm, and in the midzonal regions in addition to polymorphonuclear infiltration there were accumulations of lymphocytes with macrophages which appeared to be erythrophagocytic. Figures 2 and 2a show these changes. In another patient, G. L., a 51 per cent third degree, there were changes showing sinusoidal congestion, cloudy swelling, and minimal fatty degeneration in the central vein area. There appeared to be some increase in lymphocytes in the portal area. No focal necrosis was seen. The von Kupfer cells in some places appeared prominent. Figures 3 and 3a show the pathological changes.

That these changes occur early is indicated by the last two cases described. Both patients had severe burns and lived five and four days respectively. The necropsies were done within five hours post-mortem. The possibility that the changes observed were due to post-mortem autolysis is unlikely.

The weights and gross appearance of the liver is recorded in Table V. Generally there was not much to be observed from the gross specimens, other than slight enlargement. Gross fatty changes were not prominent.

It would appear from these observations that in addition to changes in liver function tests, there are histopathological changes in the liver. No correlation exists, and we have no biopsy data on the less severe burns. The mechanism by which these changes were produced is not evident, but speculation leads us to wonder about the absorption of products of tissue disintegration from the burned surface. Shock was prevented and we have no reason to believe that there was a diminution of blood flow to the liver to produce these changes. Hourly urine output was good and was used as an index of renal blood flow. Certainly the greater the amount of third degree burn the more marked were the changes in liver function. We would tend to agree with the statements of Gillman and Gillman (5), that if the liver plays a part in causing the death of the burned individual it does so at a level where fundamental biochemical processes involving protein synthesis, detoxification, and probably reticulo-endothelial activity are impaired. The finding of erythrophagocytosis in one patient suggests that mechanism may play a part in the anemia of thermal burns.

TABLE V

Patient	Amount of third degree	Hours post-mortem	Weight	Description
	per cent			
F. T. W. M., 30	30	12	2,350	External surface smooth; edges rounded, reddish-brown in color. Cut surface bleeds easily.
J. M. N. M., 84	38	29	1,800	Firm, smooth, light brown in color. Few pale areas in parenchyma.
G. L. N. F., 19	51	5	1,870	Bluish red, with firm consistency; internal architecture and vascular markings prominent.
F. J. N. F., 20	55	15	1,425	Surface pale yellowish and smooth; cut parenchymal surface bulges; lobular pattern distinct and generally yellowish color.
O. B. N. F., 29	65	2	1,840	Cut surface is fatty, bulges; congestion in the center of the lobule.

The early appearance of changes in liver function suggests the advisability of initiating immediate preventive therapy against liver damage whether it be due to the absorption of products of tissue damage or due to profound alterations in hepatic physiology produced by a sudden stress phenomenon. Preliminary trials by us, using dietary measure and in one patient lipotropic agents such as methionine, have been generally ineffective. By diminishing the reabsorption of urobilinogen, we thought we might remove a functional load on the liver, and so aureomycin was administered orally. We observed no beneficial effect of aureomycin in these individuals in so far as changes in the liver function are concerned.

FIG. 1

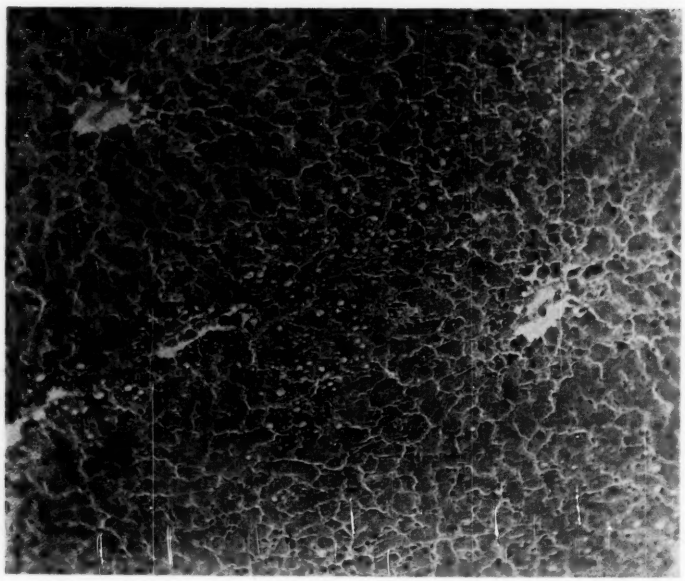

FIG. 1a

Figures 1 and 1a are taken of liver sections from patient F. T. who had 30 per cent third degree and 95 per cent total burn. Note the generalized cloudy swelling, sinusoidal congestion, and the fatty infiltration and fat lobules in the portal areas. There was marked urine urobilinogen excretion just prior to death.

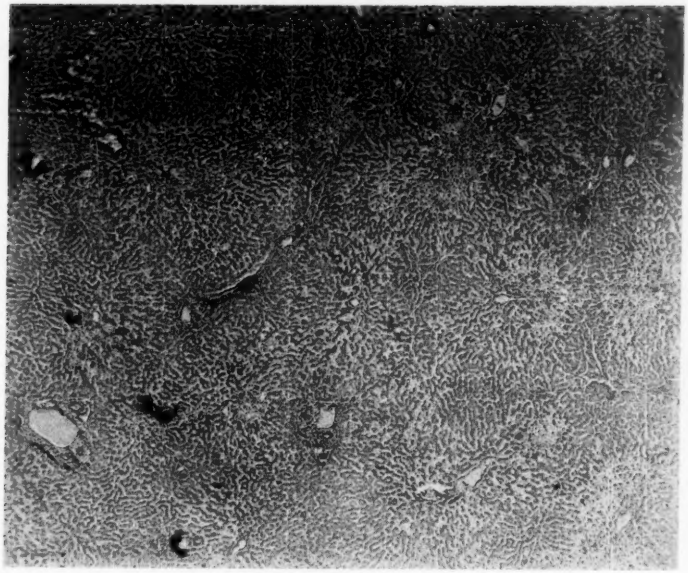

FIG. 2

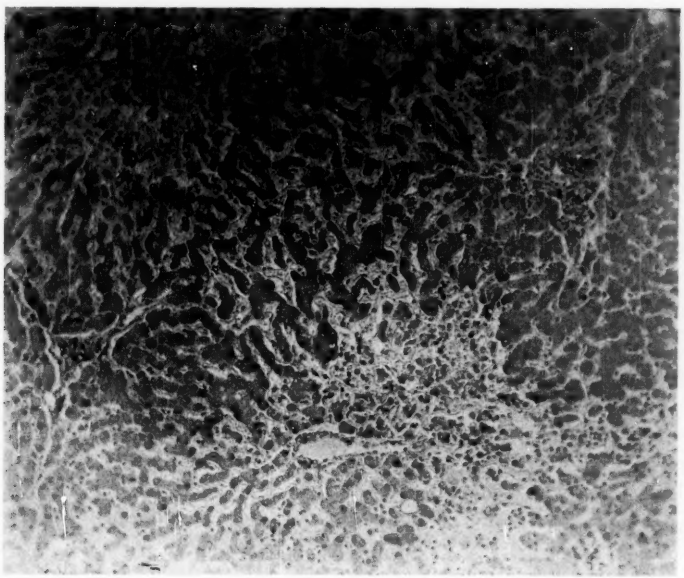

FIG. 2a

Figures 2 and 2a are taken of the liver sections from the patient O. B. with 65 per cent third degree burns. Note the marked sinusoidal congestion, areas of focal necrosis, and cloudy swelling. Eosinophilic macrophages were present and erythrophagocytosis observed. In some areas hyalinized eosinophilic bodies were seen.

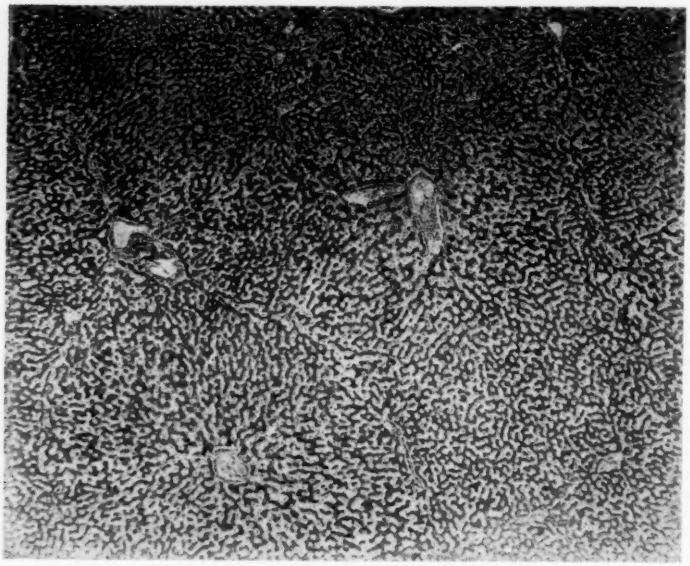

FIG. 3

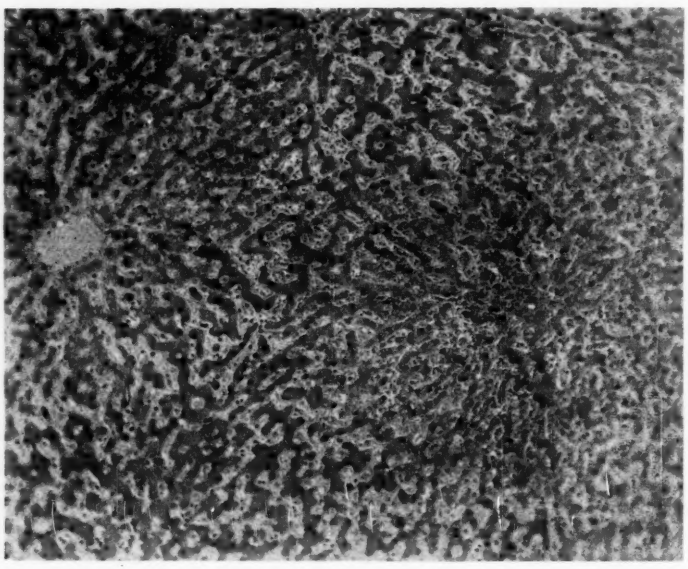

FIG. 3a

Figures 3 and 3a are from the liver sections of G. L. who suffered a 51 per cent third degree burn. There is sinusoidal congestion, cloudy swelling, and minimal fatty degeneration in the central vein areas. No focal necrosis was observed.

Two of the patients were on diets which provided very high protein and carbohydrate intakes with practically no fat. Milk protein and milk products formed the base of the diets and vitamin supplements were given in the quantities recommended by the National Research Council. The patients on this regime still manifested marked changes in the albumin-globulin ratio and in the B.S.P. retention as well as in the other liver function tests employed. We might speculate that this apparent disturbance in protein synthesis and/or excessive albumin loss probably has some part in the anemia of thermal injury through impaired globin synthesis.

CONCLUSIONS

Minor to extensive third degree burns show early impairment of the liver function as judged by changes in the liver function tests employed in this study. The more severe the burn the greater the amount of change in liver function.

One of the most constant changes was the increase in urine urobilinogen excretion and alterations in the albumin-globulin ratio, both considered to be sensitive evidences of liver function.

Autopsy material on five fatal burns showed no constant histopathological change, but there was evidence of fatty infiltration, cloudy swelling, increased pigments in the reticulo-endothelial cells, focal necrosis, and congestion in the liver substance.

More study is necessary to understand the mechanism whereby liver function failure occurs and attention should be directed at the prevention, as well as treatment, of liver dysfunction in severe burns.

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EFFECT OF DESOXYCORTICOSTERONE GLUCOSIDE UPON CEREBRAL BLOOD FLOW AND METABOLISM OF HUMAN SUBJECTS [1]

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INTRODUCTION

An effect of steroidal hormones upon cerebral metabolism was suggested by Selye's (1) observation that steroids possess anesthetic properties when administered to experimental animals. Gordan and Elliott (2) subsequently showed that certain steroids inhibit glucose utilization by rat brain *in vitro* and that their comparative inhibitory activity parallels their reported anesthetic potency. These observations were confirmed by Hayano, Schiller, and Dorfman (3). The possibility that steroidal inhibition of cerebral oxidative processes may be a physiological occurrence was demonstrated by Eisenberg, Gordan, and Elliott (4, 5). They found that deprivation of gonadal steroids by castration produces an increase in the rate of glucose utilization by rat brain and that the *in vivo* administration of testosterone, as well as other steroids with the androstene nucleus, prevents the characteristic rise. Desoxycorticosterone was the most potent of the steroidal compounds tested.

Evidence of an *in vivo* action of steroidal hormones on cerebral functions in man may be inferred from the observations of Pincus and Hoagland (6–9). In controlled studies, they found that the administration of pregnenolone to human subjects results in a significant lowering of fatigue responses to psychomotor tests. It is of interest that euphoria, depression, and exhilaration have been reported from the administration of various steroids in man (8–13).

The known anticonvulsive activity of desoxycorticosterone further suggests a steroidal influence on cerebral processes. Woodbury and Davenport (14) have demonstrated that the lowered electro-shock seizure threshold of adrenalectomized animals may be restored to normal levels, and that it may be increased above normal in intact animals by desoxycorticosterone acetate. In human epileptic patients, the anticonvulsive activity of this steroid has also been demonstrated. McQuarrie, Anderson, and Ziegler (15) and Aird and Gordan (16) have reported a reduction in the frequency of convulsive episodes of epileptic patients receiving desoxycorticosterone acetate. Desoxycorticosterone glucoside, an active water-soluble preparation of desoxycorticosterone, has been shown to reduce the electro-encephalographic abnormalities of epileptic patients (16).

These considerations have stimulated further investigation of steroidal activity upon cerebral metabolism in man. There is a suitable method, the nitrous oxide technique of Kety and Schmidt (17, 18), for the determination of cerebral blood flow in man. An estimation of the rate of cerebral blood flow permits a computation of the rate of utilization or production by the brain of any substance which can be accurately analyzed in blood. A steroidal compound well adapted for use in short-term studies in man is also available in the potent, water-soluble desoxycorticosterone glucoside (DCG).[3] The conjunction of a workable method for the estimation of the rate of cerebral blood flow and an active, water-soluble steroid has made feasible a measurement of steroidal effects on the metabolism of the human brain *in vivo*.

METHOD

Cerebral blood flow (CBF) and cerebral metabolic rate (CMR) were estimated in 15 human subjects by the original nitrous oxide method of Kety and Schmidt (17,

[1] Aided by a cooperative grant between the National Heart Institute and the University of California and a grant-in-aid from the Research Committee of the University of California.

[2] Schering Fellow in Endocrinology, 1949–1950.

[3] The desoxycorticosterone glucoside used in this study was generously furnished by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

18). Of the patients studied, seven had no endocrine disease, one had mild diabetes mellitus controlled by diet alone, two had been castrated for prostatic carcinoma, one represented Klinefelter's syndrome with hypoleydigism, and four had chromophobe adenomas with secondary hypogonadism. The subjects received no medications for 24 hours or more before the study and hormonal medications had been withheld for the preceding two or more weeks.

With the fasting subject supine, needles were inserted into the jugular bulb and either the femoral or internal carotid artery and connected to heparinized manifolds as described by Kety and Schmidt. In order to avoid the effects of emotional stimuli, as stressed by Scheinberg and Stead (19), no further procedures were undertaken until a steady state was achieved as determined by stable cardiac rate and blood pressure. In 14 of the 15 patients, two determinations of the rate of cerebral blood flow were made consecutively. Twenty minutes were allowed to elapse after the control determination to permit expiration of nitrous oxide, and a second blood blank for nitrous oxide was obtained in each case. Prior to proceeding with the second cerebral blood flow determination, specimens for control glucose levels of arterial, cerebral venous and, in seven cases, peripheral (antecubital) venous blood were obtained. All of these were drawn simultaneously at two-minute intervals until three separate sets of control specimens had been obtained.

Immediately after the third set of control samples for glucose determination were taken, 50 mgm. of DCG were injected intravenously and a second determination of the cerebral blood flow was made without delay. Blood sugar values were determined on aliquots of the simultaneously drawn experimental specimens of arterial and cerebral venous blood throughout the procedure for comparison with the previously drawn control specimens. Following completion of the second cerebral blood flow determination, two additional sets of specimens were obtained for analysis of sugar levels at three-minute intervals. When peripheral (antecubital) venous specimens were obtained, they were drawn simultaneously with the arterial and cerebral venous specimens. During each cerebral blood flow determination, arterial and cerebral venous oxygen and carbon dioxide content was determined upon separate specimens consisting of 5 cc. of blood drawn immediately before the first CBF sample and an equal amount drawn into the same syringe directly following the five-minute CBF specimen.

Blood oxygen and carbon dioxide analyses were made in duplicate by the manometric technique of Van Slyke and Neill (20), as modified by Kety and Schmidt (18). Blood sugar content was determined by the iodometric titration method of Somogyi (21, 22) and yielded results reproducible within 1 per cent. In accordance with the injunction of Somogyi (23) that, when dealing with changes in arterio-venous glucose difference, accurate analytical methods must be selected and employed with meticulous care, all glucose determinations were performed in duplicate by one of us (R.C.B.). Mean arterial blood pressure was obtained directly from the femoral or carotid artery by means of an air-damped mercury manometer connected to the arterial manifold. Cerebral oxygen consumption, cerebral glucose consumption or production, and cerebral carbon dioxide production were calculated from the rate of cerebral blood flow by the formulae of Kety and Schmidt.

EXPERIMENTAL DATA

Experimental data are presented in Table I. For each function measured, the mean value and the standard deviation of the mean is included.

Striking and rapid effects were noted upon blood sugar levels following administration of 50 mgm. of DCG. Uniformly, the cerebral venous glucose concentration rose significantly—in eight instances above the arterial level—effecting in 13 of the 15 cases a reduction in the cerebral arterio-venous glucose difference. While the individual changes in the arterial and peripheral (antecubital) venous blood sugar concentrations were inconsistent and not marked, a small, but statistically significant ($p < 0.01$), rise occurred in both the mean arterial and the mean antecubital venous blood sugar concentrations following the administration of DCG. Examples of the glucose concentration curves are shown in Figures 1 and 2. It should be noted that the changes in glucose concentration occurred without alteration in the rate of cerebral blood flow. Figure 3 depicts the change in mean cerebral arterio-venous glucose difference produced by DCG. This demonstrates

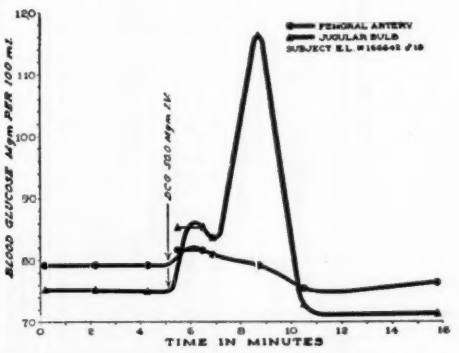

FIG. 1. EFFECT OF DCG ON CEREBRAL VENOUS AND ARTERIAL GLUCOSE CONCENTRATIONS

Note that the cerebral venous sugar level rises rapidly above the arterial glucose level and remains elevated for approximately five minutes.

TABLE I

The effect of desoxycorticosterone glucoside (DCG) on cerebral blood flow and metabolism

No.	Subject	Sex	Age	Diagnosis	CBF* cc./100 gm./min.		MABP† mm. Hg.		Cardiac rate per minute		A-V_{oxygen} Vol. per cent		CMR_{oxygen} cc./100 gm./min.		A-V_{CO_2} Vol. per cent		CMR_{CO_2} cc./100 gm./min.		Maximum A-CV‡ glucose‖ mgm. per cent		$CMR_{glucose}$ mgm./100 gm./min.		Maximum A-PV§ glucose‖ mgm. per cent		Maximum change in glucose after DCG¶ mgm. per cent		
					Control	DCG	Control	DCG	Control	DCG	Control	DCG	Control	DCG	Control	DCG	Control	DCG	Control	DCG	Control	DCG	Control	DCG	CV	Arterial	PV
1	M. C.	F	35	No endocrine disease	73	33	78	72	76	76	4.5	4.4	3.3	1.5	3.6	2.9	3.4	1.0	+ 4	−19	+ 3	− 6	+ 5	+ 1	↑ 26	↓ 2	↑ 5
2	H. R.	M	17	No endocrine disease	37	27	68	68	75		5.0	6.4	1.9	1.7	3.4	3.2	1.3	0.9	+ 4	− 5	+ 1	− 1			↑ 11	↑ 7	
3	D. A.	F	46	No endocrine disease	52	36	105	105	94	90	7.5	7.8	3.9	2.8	7.0	7.2	3.6	2.6	+ 9	− 3	+ 4	− 1			↑ 8	↓ 4	
4	D. P.	M	48	No endocrine disease	47	36	105	105	60	60	7.8	7.6	3.7	2.7	7.6	5.8	3.6	2.1	+13	− 9	+ 6	− 3	+12	+10	↑ 22	0	↑ 2
5	W. W.	M	29	No endocrine disease	109	86	58	58	64	64	5.4	5.7	5.9	4.9	3.8	3.7	3.4	3.2	+ 7	+ 3	+ 8	+ 3	+16	+14	↑ 10	↑ 6	↑ 6
6	F. N.	M	44	No endocrine disease	56	75	158	162	82	80	4.7	5.0	2.6	3.8	3.3	5.1	1.9	3.8	+ 6	+ 1	+ 3	+ 1	+ 6	+ 6	↑ 3	↑ 8	↑ 2
7	H. H.	M	53	Diabetes mellitus	38	32	60	60	84	84	9.4	6.5	3.6	2.1	9.3	7.0	3.5	2.2	+26	+17	+10	+ 5			↑ 3	↓ 6	
8	L. D.	M	34	Chromophobe adenoma with secondary hypogonadism	60	78	82	86	70	72	6.4	5.6	3.8	4.4	6.1	5.9	3.7	4.7	+ 6	− 2	+ 3	− 1			↑ 4	↓ 4	
9	M. R.	F	51	Chromophobe adenoma	53		90	90	80	74	2.0	3.6	1.1		2.9	4.8	1.5		+ 3	+ 2	+ 2				↑ 1	↑ 3	
10	L. L.	M	35	Chromophobe adenoma							6.6	6.9			5.7	5.6			+ 4	+ 4			+ 1	+ 4	↑ 5	↑ 5	↑ 2
11	E. L.	M	18	Chromophobe adenoma with secondary hypogonadism	57	65	65	68	80	80	5.1	3.5	2.9	2.3	5.6	6.5	3.2	4.2	+ 4	−38	+ 2	−25			↑ 42	↓ 1	
12	R. W.	M	22	Klinefelter's syndrome with hypoleydigism	71	83	78		80		4.8	3.7	3.4	3.1	3.3	3.4	3.1	2.9	+ 6	− 9	+ 4	− 8			↑ 24	↑ 9	
13	W. P.	M	49	Primary testicular eunuchoidism	48	41	65	69	66	68	5.3	4.8	2.5	2.0	3.6	2.8	1.7	1.2	+ 3	+ 5	+ 1	+ 2	+ 5	+10	↑ 6	↑ 8	0
14	J. B.	M	69	Castrate (carcinoma of prostate)	62	61	98	98	62	62	3.3	5.9	2.0	4.2	3.8	7.0	2.4	4.3	+ 3	− 6	+ 2	− 3			↑ 19	↑ 10	
15	A. H.	M	64	Castrate (carcinoma of prostate)	55	57	150	135	83	80	4.6	4.9	2.5	2.8	3.9	5.2	2.1	2.9	+12	+ 9	+ 7	+ 5	+ 4	+ 4	↑ 10	↑ 7	0
Mean of all observations					58	55	90	91	75	74	5.5	5.5	3.1	2.8	4.9	5.1	2.7	2.8	+ 7.2	− 3.3	+ 4.1	− 2.6	+ 6.8	+ 6.9	↑ 13.3	↑ 3.1	↑ 3.9
Standard deviation of the mean					± 5	± 6	± 9	± 9	± 3	± 3	±0.5	±0.4	±0.3	±0.3	±0.5	±0.4	±0.2	±0.4	± 1.6	± 3.5	± 0.8	± 1.3	± 2.0	± 1.8	± 3.0	± 1.3	±0.8

* CBF = Cerebral blood flow in cc./100 gm. of brain/minute.
† MABP = Mean arterial blood pressure.
‡ CV = Cerebral venous blood from internal jugular bulb.
§ PV = Peripheral venous blood from antecubital vein.
‖ Positive figure indicates arterial concentration greater than venous; negative figure indicates venous concentration greater than arterial.
¶ ↑ indicates increase after DCG.
↓ indicates decrease after DCG.

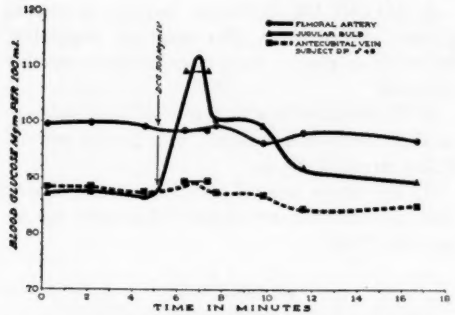

FIG. 2. EFFECT OF DCG ON SUGAR CONTENT OF ARTERIAL AND ON CEREBRAL AND PERIPHERAL VENOUS BLOOD

Note prompt rise of cerebral venous sugar level above arterial glucose concentration in contrast to stable peripheral venous glucose level.

an increase of the mean cerebral venous glucose concentration above the mean arterial glucose concentration after administration of DCG, and, in conjunction with the statistically significant change in $CMR_{glucose}$, is interpreted to indicate liberation of glucose from the brain.[4]

The mean rates of cerebral blood flow, oxygen consumption, and carbon dioxide production did not change significantly. As previously noted by Green and his associates (24), DCG did not alter the mean arterial blood pressure or cardiac rate. No immediate or subsequent clinical effects were apparent.

DISCUSSION

These experimental findings lend the support of quantitative measurements in man to the thesis that steroidal hormones may affect cerebral carbohydrate metabolism. The consistently observed increase in cerebral venous glucose concentration, with a decrease in the cerebral arterio-venous glucose difference produced by DCG in the absence of increased cerebral blood flow, could have resulted from either diminished cerebral utilization of glucose, liberation of sugar by the brain, or both. An increase in the sugar concentration of the cerebral venous blood above that of arterial blood obtained simultaneously indicates liberation of sugar by the brain. From these observations alone, however, it is not possible to conclude that there is not also an alteration of the cerebral utilization of glucose.

From the present data, the mechanism and specificity of this effect may be only conjectured. Of the several possible sources for the sugar liberated from the brain following DCG, a derivation from intracellular glycogen stores may be considered. Kerr and Ghantus (25) have demonstrated sufficient quantities of glycogen in the brains of dogs and rabbits to account for the amount of sugar "liberated" following injection of DCG. The presence of some endogenous substrate in human brain which can be utilized for cerebral oxidative processes *in vitro* is suggested by Elliott, Sutherland, and Boldrey (26). They found that the oxygen consumption of slices of human cerebral cortex was maintained for a significantly longer period in the absence of a glucose substrate than was the oxygen consumption of rat brain, which dropped off rapidly. If this substrate is glycogen, the glycogenolytic potentiality of desoxycorticosterone reported by Verzar and Wenner (27) would be in harmony with our observations.

Other sources must also be considered, among them the intracellular and extracellular glucose

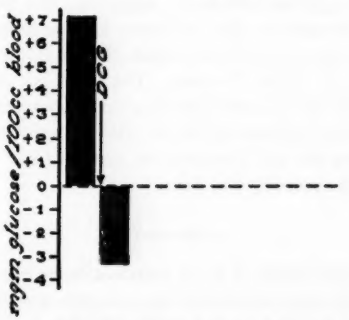

FIG. 3. CEREBRAL ARTERIO-VENOUS GLUCOSE DIFFERENCE: MEAN VALUES FOR 15 SUBJECTS BEFORE DCG AND AT THE PEAK RESPONSE AFTER DCG

Positive value before DCG indicates that the arterial glucose concentration is higher than the cerebral venous glucose level (glucose utilization). Negative value after DCG indicates that the cerebral venous glucose level exceeds the arterial concentration (glucose "liberation").

[4] As performed, the Somogyi method is a measure of reducing substance content, almost exclusively monosaccharides. Subsequent to the preparation of this report, we have found that the bulk of the reducing substance liberated into the cerebral venous blood is non-fermentable, and preliminary attempts at identification suggest galactose.

and cerebral galactosides, but data regarding these are not yet available. Further studies to extend these observations and their specificity are in progress.

No significant alteration in the mean CMR_O, or CMR_{CO}, was observed, indicating that desoxycorticosterone glucoside does not influence the aerobic phase of cerebral energy metabolism as an immediate general effect, although it may alter the substrate. However, it is interesting that the cerebral metabolic rate was diminished following administration of DCG in all but one ortho-steroidal subject and that no change or only a slight increase in cerebral metabolic rate was apparent in all hyposteroidal subjects. Until the individual groups are expanded, it is not possible to establish the significance of this observation. In this connection it is recognized that the data included in the present paper group together observations made upon individuals with divergent endocrine states. The data suggest that enlargement of the groups may eliminate some of the variability due to the lumping together of non-comparable individuals. The fact that the standard deviations of the mean in this study are somewhat greater than those reported by other workers may, in part, be explained on this basis.

While the arterio-venous sugar difference reflected in the cerebral circulation is altered, no such change occurs in the peripheral arterio-venous glucose difference, suggesting the absence of an immediate effect of desoxycorticosterone glucoside upon the glucose metabolism of the muscular tissues of the forearm. The small but significant rise in the mean arterial glucose concentration following administration of DCG might be accounted for by liberation of glucose from other sources, possibly the liver.

SUMMARY

1. The effects of desoxycorticosterone glucoside upon the cerebral utilization of oxygen and glucose and the production of carbon dioxide in human subjects has been studied by the nitrous oxide technique of Kety and Schmidt.

2. Administration of DCG produced a rise in the cerebral venous sugar concentration above the arterial level, indicating that this compound causes liberation of sugar from the brain.

3. A small, but significant, increase in arterial glucose concentration also occurred, suggesting liberation of glucose from other sources, possibly the liver.

4. Preliminary data suggest a different response to DCG between individuals with normal and deficient steroidal status.

5. The mean arterial blood pressure, cardiac rate, and rate of cerebral blood flow were not altered by DCG.

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THE RELATION BETWEEN PROTEIN CONSUMPTION AND DIURNAL VARIATIONS OF THE ENDOGENOUS CREATININE CLEARANCE IN NORMAL INDIVIDUALS [1]

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In a previous paper based on the same study (1), it was shown that the serum urea concentration of subjects with intact renal function increases with protein consumption. In contrast, the serum creatinine concentration in such subjects was shown to be independent of protein consumption (2). It has long been known that endogenous urea excretion increases with the protein consumed, while the rate of endogenous creatinine excretion has been shown to be relatively independent of the amount of protein consumed (3).

Popper and Mandel (4) first utilized the endogenous creatinine clearance in man as a measure of the glomerular filtration rate. Steinitz and Türkand (5) found a good correspondence between the inulin and endogenous creatinine clearances in normal subjects. More recently Brod and Sirota (6) have studied the endogenous creatinine chromogen/inulin clearance ratio in man, and concluded that the endogenous creatinine clearance corresponds closely to the inulin clearance in normal subjects. Discrepancies which appear in subjects with renal disease and markedly impaired renal function, are not sufficient to affect seriously the clinical value of the test as a general indicator of renal competence. This judgment has been confirmed by Camara (7), as well as by numerous clinical observations of our own.

Miller and Dubos (8) showed that creatinine is not the only chromogen in human serum that produces color in the Jaffé reaction. The ratio of such "pseudocreatinine" to creatinine is higher in azotemic individuals. Brod and Kotátko (9) found that the endogenous creatinine and the inulin clearances approach identity only when the "true" creatinine clearance is used. They used Lloyd's reagent to adsorb creatinine and determined the residual "pseudocreatinine" concentration in the serum.

Diurnal variations in the endogenous creatinine clearance have been studied by Brod (10). He found that the endogenous creatinine clearance in normal individuals is depressed during the night. In a more complete investigation, Sirota, Baldwin and Villarreal (11) studied diurnal variations in renal function of normal male subjects. They found no significant difference in the night and day values for the endogenous creatinine clearance, except for a slight fall in the period from 12 Midnight to 4 A.M. These experiments were conducted on "routine ward" diets, and no attempt was made to vary or to control at a constant level the consumption of protein or of creatinine-containing foods.

Of all renal function tests, the endogenous creatinine clearance is easiest to perform since no foreign substance is infused, the serum concentration is constant, and urine collections can be made over long periods of time, up to 24 hours, thus minimizing the errors in collection. Those who have struggled with the methods for performing inulin determinations realize the relative precision and simplicity of the creatinine determination, as modified by Bonsnes and Taussky (12). For these reasons it seemed desirable to investigate the effect of dietary protein consumption upon the endogenous creatinine clearance and its diurnal variation, since this matter has not been considered in the previous publications.

A clinical test is of optimal usefulness if its performance is simple. For this reason, because we were concerned with the endogenous creatinine clearance as an indicator of renal competence without regard to the possible analysis of indi-

[1] This work, performed in 1945–46, was made possible by a grant from the Nutrition Foundation, Inc.

[2] Dr. Addis died June 4, 1949.

[3] Fellow of the John Simon Guggenheim Memorial Foundation.

TABLE I

Diurnal variation of serum creatinine concentration *in relation to dietary protein consumption in normal male individuals*

Diet	7:15 A.M.			11:45 A.M.			4:45 P.M.			9:45 P.M.		
g. protein/kg.	no. obs.	mg. %	stand. dev. mean	no. obs.	mg. %	stand. dev. mean	no. obs.	mg. %	stand. dev. mean	no. obs.	mg. %	stand. dev. mean
0.5	26	1.02	±0.08	34	1.03	±0.09	17	1.08	±0.11	17	1.06	±0.06
1.5	18	1.00	±0.09	18	1.01	±0.09	8	1.05	±0.07	9	1.09	±0.07
2.5	34	1.04	±0.07	36	1.04	±0.07	17	1.04	±0.07	17	0.99	±0.09

vidual renal functions, it was not considered desirable to complicate the determinations by the use of Lloyd's reagent. In addition, the test is most useful in evaluating patients with slight degrees of renal functional impairment, before the development of azotemia. In such patients the "pseudocreatinine" chromogen is at a minimum.

METHOD

1. *Conditions of the experiment*

The conditions have been previously described in detail (1, 2). The principal subjects in this study were nine male internes. They were fed carefully contrived diets, all providing more than 2,000 calories per day, with fluid consumption permitted *ad libitum*. From Monday breakfast (7 A.M.) until Saturday at noon the diet was carefully followed. Over the weekend, until the following Monday at breakfast, the subjects were allowed a free diet. The breakfasts were free of pre-formed creatinine; that is, they contained no meat, fish, or chicken. At other meals meat was used as needed to provide the desired amount of protein. During the first week the diet contained 0.5 g. protein per kg. body weight per day. During the second week the protein content of the diet was 1.5 g. per kg. body weight, and during the third week the protein content was raised to 2.5 g. per kg. body weight. After a gap of two months, the experiment was repeated at lowest and highest levels of protein consumption.

Urine collections and blood specimens were obtained on the last two days of each diet. Friday was divided into four urine collection periods; 7 A.M. to 12 Noon, 12 Noon to 5 P.M., 5 P.M. to 10 P.M. and 10 P.M. to 7 A.M. Blood specimens were obtained just before each meal and at bedtime, with a 15 minute leeway allowed in either direction from the times indicated: 7:15 A.M., 11:45 A.M., 4:45 P.M. and 9:45 P.M. On Saturday there was a single urine collection from 7 A.M. to 12 Noon, while blood specimens were obtained at 7:15 A.M. and 11:45 A.M.

In order to compare the normal clearance values in women with those in men, a group of 25 female nurses, dietitians and medical students were studied in a similar fashion. However, the experiment was modified for this group and they were studied only on the 0.5 g. protein diet. Urine collections were made each day of the week from 10 P.M. to 7 A.M., while blood specimens were taken daily at 3:15 P.M.

2. *Methods of Measurement*

Creatinine determinations were performed by the Folin-Wu method, as modified (2). Clearances were calculated from the measured excretion rate, expressed in mg. per 24 hours, corrected to a body weight of 70 kg. The serum concentration used in the calculation was a logarithmic mean of the concentrations found at the beginning and the end of each period. For body weight corrections, the subjects were weighed daily. Clearance values were corrected by a factor $\frac{70^{0.7}}{BW^{0.7}}$, where BW = body weight in kg., in accordance with the suggestion of Addis (13). The calculated clearances were expressed in L. per 24 hours per 70 kg. body weight.

RESULTS

As has previously been shown (2), the serum creatinine concentration does not vary significantly with diet in normal individuals. From Table I it is seen that the diurnal variation in serum cre-

TABLE II

Diurnal variation of endogenous creatinine excretion *in relation to dietary protein consumption in normal male individuals*

Diet	7 A.M.–12 Noon			12 Noon–5 P.M.			5 P.M.–10 P.M.			10 P.M.–7 A.M.		
g. protein/kg.	no. obs.	mg./24 hrs./70 kg.	stand. dev. mean	no. obs.	mg./24 hrs./70 kg.	stand. dev. mean	no. obs.	mg./24 hrs./70 kg.	stand. dev. mean	no. obs.	mg./24 hrs./70 kg.	stand. dev. mean
0.5	31	1760	±164	16	1783	± 77	16	1823	±119	16	1517	±97
1.5	16	1858	±112	9	1896	± 81	9	1935	± 83	9	1611	±78
2.5	33	1844	±155	14	1988	±135	18	2081	±150	17	1687	±90

TABLE III

Diurnal variation of endogenous creatinine clearance *in relation to dietary protein consumption in normal male individuals*

Diet	7 A.M.–12 Noon			12 Noon–5 P.M.			5 P.M.–10 P.M.			10 P.M.–7 A.M.		
g. protein/kg.	no. obs.	L./24 hrs./70 kg.	stand. dev. mean	no. obs.	L./24 hrs./70 kg.	stand. dev. mean	no. obs.	L./24 hrs./70 kg.	stand. dev. mean	no. obs.	L./24 hrs./70 kg.	stand. dev. mean
0.5	24	171	±17	16	167	±15	16	166	±15	16	141	±12
1.5	16	180	±17	8	177	±10	8	179	±14	9	151	±15
2.5	31	178	±17	14	182	±17	16	200	±14	16	164	±13

atinine concentration is insignificant, with a wide range of protein consumption, even though the consumption of pre-formed creatinine was limited to the noon and evening meals.

As seen in Table II, on each diet there is a moderate but significant drop in creatinine excretion during the period from 10 P.M. to 7 A.M. In addition, there is a slight but significant rise in creatinine excretion during all periods, except 7 A.M. to 12 Noon, as the dietary protein consumption rises. Since the serum creatinine concentration remains constant, the clearance values follow the variations in excretion, and this may be seen in Table III.

The female subjects were observed only under one set of conditions, so that effects of diet and diurnal variation were not noted. However, Table IV reveals that both the serum creatinine concentration and the creatinine excretion were lower than the values obtained in males on the same diet and at the same times. In spite of this the mean clearance value was not significantly different from the value obtained in males under similar conditions.

DISCUSSION

Since there was a drop in creatinine excretion during the period from 10 P.M. to 7 A.M. and there was a rise during the period from 7 A.M. to 12 Noon, after a creatinine-free breakfast, on all diets, the factor responsible for the nocturnal drop must be other than the dietary protein consumption. In view of the observations of others that various renal functional measurements rise during recumbency and drop when the erect, lordotic posture is assumed (14–16), it seems improbable that the nocturnal drop in creatinine excretion is a postural effect. Likewise, it seems improbable that this nocturnal drop is the result of diminished physical activity, since several investigators have found that renal functional measurements made at rest are greater than those made during mild exercise and much greater than those made during severe exercise (17–19).

TABLE IV

Nocturnal creatinine clearance values in normal females on a low protein diet (0.5 g. per kg. per day)

	No. obs.	Value	Stand. dev. mean
Serum creat. conc. (3 P.M.)	124	0.79 mg. %	±0.07
Creat. excret. (10 P.M.–7 A.M.)	124	1147 mg./24 hrs./70 kg.	±94
Creat. clear. (10 P.M.–7 A.M.)	124	146 L./24 hrs./70 kg.	±14

During sleep a much greater degree of relaxation is attained than during rest while awake. There is a generalized relaxation of the vascular bed, manifested by the drop in vascular tension that occurs during sleep in normal individuals. It seems most reasonable to suppose that the nocturnal drop in creatinine excretion, and consequently in the endogenous creatinine clearance, is the result of such a relaxation of the vascular tension, with a drop in filtration pressure at the glomerulus and drop in the glomerular filtration rate. In this connection it is of interest to note that Sirota and his associates (11), by using shorter nocturnal urine collection periods, found the period of diminished inulin and endogenous creatinine clearances to coincide with the period of deepest sleep.

The influence of diet upon the endogenous creatinine clearance is absent in the morning period from 7 A.M. to 12 Noon, after a creatinine-free breakfast. From this it is apparent that the fasting period from supper time until morning is sufficient to obtain basal conditions with respect to creatinine excretion. For this reason, it seems most desirable for clinical determinations to use the morning period for collection, after a creatinine-free breakfast. This simply means a normal break-

fast of eggs, toast, cereal, etc., but without meat or bacon.

In this experiment, there was no significant variation in the serum creatinine concentration of the male subjects under any of the varied conditions, with a grand mean value of 1.03 mg. per 100 ml. Therefore, it would seem satisfactory to use a serum specimen obtained just before, during, or just after the period of urine collection for calculation of the clearance.

In the female subjects the serum creatinine concentration was slightly, but significantly, lower than in the males, with a mean value of 0.79 mg. per 100 ml. However, the creatinine excretion was proportionately lower than in the males, so that the mean endogenous creatinine clearance did not differ significantly from the value obtained in males under the same conditions.

The values obtained for the endogenous creatinine clearance during the morning collection period from 7 A.M. to 12 Noon had a mean value of 176 L. per 24 hours per 70 kg. body weight. This is almost identical with the values obtained by Sirota and his colleagues (11) for the "peak," when translated into comparable units.

SUMMARY

The endogenous creatinine clearance is a valuable, simple, clinical test of renal function. It is affected slightly by the dietary protein consumption and by diurnal variations. These influences may be eliminated, for practical purposes, by collecting urine for the test during the morning after a creatinine-free breakfast. The normal endogenous creatinine clearance under such conditions is 176 L. per 24 hours per 70 kg. body weight, or 122 ml. per minute per 70 kg. body weight.

In females the normal mean serum creatinine concentration is 0.79 mg. %, slightly lower than in males. The normal mean endogenous creatinine clearance for female subjects was determined under one set of conditions and did not vary significantly from the values obtained in males.

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THE CARDIAC OUTPUT IN PATIENTS WITH ARTERIOVENOUS FISTULAS[1]

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The study of arteriovenous fistulas, and the alterations in local and systemic circulation which they produce, has been a means of not only understanding this particular lesion better, but of extending our knowledge of many other more common circulatory phenomena. In peacetime, investigations have been conducted on the relatively rare clinical cases and on animals with fistulas produced in the laboratory. The recent war, with its many vascular injuries, presented an unusual opportunity to study the circulatory alterations in patients with arteriovenous fistulas. This and succeeding reports deal with observations on a large group of patients treated at Ashford General Hospital.

The clinical manifestations exhibited by patients with an arteriovenous fistula have been described elsewhere (1, 2). It is generally recognized that these patients may develop congestive heart failure. There has been controversy, however, regarding the mechanism producing the circulatory failure. Some have ascribed it to greatly increased demands on the heart, others to diminution of coronary blood flow secondary to the lowered mean arterial pressure (3). Since a knowledge of the output of the heart is so important in defining the mechanism of the altered circulatory dynamics in these patients, we have measured the cardiac output as part of a general study of the circulation in patients with arteriovenous fistulas.

In 1923 Lewis and Drury (4) reported the results of their studies of arteriovenous fistulas in both man and the experimental animal. In the latter, using a glass cardiometer to enclose the heart, they found an increase in cardiac output, in some instances, when the fistula was open. In the animals with an elevated cardiac output they also noticed an increased central venous pressure. Because the venous pressure was found to be normal in their human cases, they assumed that the cardiac output was normal. No actual measurements on humans were reported. Harrison, Dock and Holman (5) in 1924, using the direct Fick technique, reported that the cardiac output was elevated in their animals with experimental arteriovenous fistulas. Since then, several additional studies in experimental animals have confirmed the earlier observations that the basal cardiac output may be elevated by the creation of an arteriovenous fistula (3, 6–8). On the other hand, the relatively few clinical observations made on patients with arteriovenous fistulas led to less definitive conclusions; in most instances an increased cardiac output was found (9–14). These data have been summarized in two recent reports (3, 14). It may be that the foreign gas methods, often used in McMichael clinical studies, were invalidated by the abnormal circulatory dynamics present in these patients. Insofar as we are aware, there is no study reported of the cardiac output in a large group of patients observed before and after removal of an arteriovenous fistula.

METHODS

The cardiac output was determined by means of the low frequency, critically damped ballistocardiograph (15, 16) which measures the output by the ballistic recoil of the body with each heart beat.

To verify the validity of the measurements by the ballistocardiograph in the presence of arteriovenous fistulas or large areas of reactive hyperemia (in reality a temporary arteriovenous fistula) comparative studies by the direct Fick technique were made (16–19). A satisfactory correlation between the two methods was demonstrated. We believe, therefore, that, under the conditions of the present study, the ballistocardiographic method is a useful and valid one.

[1] The work described in the paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine, Atlanta, Georgia.

To facilitate comparison of individuals of various sizes, the cardiac index (the output of the heart in liters per minute per square meter of body surface) was used in recording our observations. All determinations were made on subjects three hours or more after they had eaten, and after they had relaxed for at least 15 minutes on the ballistocardiograph table. The arterial pressure was obtained with an ordinary mercury manometer by the auscultatory method. In patients with a fistula sufficiently distal on an extremity so that occlusion could be accomplished by inflation of a blood pressure cuff, observations were made before and during the occlusion of the fistula. These studies are reported separately (20). The transverse diameter of the heart as measured from a teleoroentgenogram was used as an index of changes in heart size. The blood volume was measured by means of the blue dye T-1824 (21).

RESULTS

Forty-seven patients with arteriovenous fistulas were studied at least once before and after operation. Many had a cardiac output determination on several occasions. All the patients were presumably normal prior to their injury resulting in the establishment of the arteriovenous fistula. All except on were men. The time between the injury causing the fistula and the operative repair varied from two months to slightly over two years. No patient had evidence of frank congestive heart failure. All the patients had definite local signs of the fistula, such as thrill or murmur. In all, the fistula was demonstrated and successfully removed at operation. In none was there evidence of recurrence, nor was the postoperative course seriously complicated.

The results of the cardiac output determinations and related observations are recorded in Table I. We have considered the value, or average of several values, obtained 10 days or more after removal of the fistula, as the normal resting output for each patient. Using this normal value for comparison, the preoperative cardiac outputs are seen to range from 127 per cent above to 21 per cent below the normal resting cardiac output (Figure 1). Our experience has been that in normal subjects a variation of as much as 25 per cent above or below a value selected as the resting cardiac output may be expected on repeated measurements on different days.

As can be seen from Table I, the alteration in cardiac output is predominantly the result of change in stroke volume rather than pulse rate.

TABLE I

Observations on 47 patients with arteriovenous fistulas before and after operation

Location of fistula and number of patients in group	Duration of fistula	Time of observation	Pulse rate	Stroke volume	Cardiac index	Change in heart size	Change in blood volume
	months	*days before and after operation*	*beats per min.*	*cc.*	*liters per min. per sq. m.*	*cm.*	*cc. per sq. m.*
Carotid (*5*)	4.9	6 before 14 after	73 70	116 80	4.5 3.7	−0.5	−440
Vertebral (*1*)	8	1 before 18 after	81 78	114 93	4.5 3.7	−0.2	
Subclavian (*3*)	3.3	19 before 4 after	71 71	114 80	4.0 2.8	+1.2	−155
Axillary (*3*)	11.3	13 before 14 after	65 78	145 104	4.9 4.5		−200
Brachial (*4*)	5	5 before 14 after	74 66	122 94	4.3 3.9	−0.2	+ 40
Iliac (*3*)	7.2	15 before 20 after	84 72	115 80	7.1 3.2	−0.6	−453
Femoral (*12*)	5	8 before 16 after	82 73	115 94	6.0 3.9	−1.2	−304
Popliteal (*5*)	9.6	9 before 21 after	66 65	134 103	4.8 3.6	−0.6	−210
Tibial (*11*)	8.2	6 before 23 after	67 70	107 94	4.0 3.5	−0.2	−123

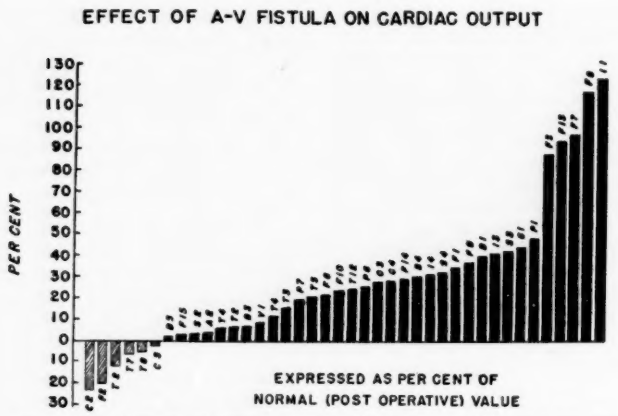

FIG. 1. THE EFFECT OF REMOVAL OF AN ARTERIOVENOUS FISTULA ON THE CARDIAC OUTPUT

In only seven patients with the fistula intact was the basal pulse rate above 85 beats per minute. In four of these the fistula involved the femoral vessel; in two, the iliac; and in one, the subclavian. The cardiac output in this group of patients averaged 82 per cent above the postoperative level and ranged from 32 per cent to 127 per cent.

The arterial blood pressure showed little variation from the preoperative to postoperative period. In general, if change occurred there was a tendency toward diminution of the pulse pressure postoperatively, due predominantly to an elevation of the diastolic pressure.

In those instances where satisfactory measurements were obtained, the changes in transverse diameter of the heart and blood volume are recorded. These are discussed in detail elsewhere (21).

DISCUSSION

Our patients were apparently healthy young adults before injury. Although the person to person variability in cardiac output is large, our study could have been made using an average normal value for comparison with the value found in the presence of the fistula. We were fortunate, however, in that our patients returned to a normal state after operation without evidence of residual abnormality in the circulation. The postoperative cardiac index varied considerably from patient to patient, but the range was similar to that found in a comparable group of normal subjects studied by the catheter method (22). Therefore, we have utilized the value obtained postoperatively for each individual as his normal and have used this value as the basis of estimating the load placed on the heart by the fistula.

The resting cardiac output with the fistula intact varied from 21 per cent below the postoperative value to 127 per cent above. A variation of 25 per cent above and below the postoperative value might be expected in any group of patients so studied and, therefore, could not be attributed to the effect of the fistula. In 22, or 47 per cent, of the patients, the change in cardiac output fell within this $\pm$ 25 per cent range. In these patients the fistula was not large enough to produce a significant change in cardiac output. In all of the other patients (25, or 53 per cent), the cardiac output with the fistula open was more than 25 per cent greater than the postoperative cardiac output, the values ranging from 25 per cent to 127 per cent above the normal output. We believe that these increases of more than 25 per cent in cardiac output are significant. This indicates that about one-half of our patients with arteriovenous fistula had a significantly elevated cardiac output as measured under basal conditions.

The increased cardiac output was primarily the result of an increase in stroke volume. The postoperative basal pulse rate was above 85 beats per minute in only seven patients. In these patients, all with clinical evidences of a large fistula, the cardiac output was markedly elevated, averaging 82 per cent above the postoperative level.

A clinical or pathological determination of the functional size of the fistula was difficult. It may be that the increase in cardiac output is the best index of the load placed on the circulation by the fistula (13). Such signs as the size of the vessel involved or the intensity of the thrill or murmur were not entirely reliable in predicting the increase in cardiac output. In general, it appeared that those patients with clinical evidence suggestive of a large fistula or one located in a large vessel had a markedly elevated cardiac output. A review of the cases studied shows that while all of those patients with a marked elevation of cardiac output had fistulas involving relatively large vessels, there appeared to be no more specific relationship. In other words, a fistula of the femoral vessel might cause either a small or a large increase in cardiac output. On the contrary, a fistula fed by a small vessel, because of the limited arterial inflow, caused at most only a small increase in cardiac output.

A correlation of the age of the fistula with the increase in cardiac output showed that most patients with a large increase had fistulas of relatively short duration; those with small increases, in many instances, had fistulas of much longer duration. However, these findings are probably due to the fact that while small fistulas may have escaped notice for a long time, the larger ones were diagnosed and referred to our hospital with greater rapidity.

It is well recognized, however, that patients with an arteriovenous fistula may have cardiac enlargement, as evidenced by an increased transverse diameter of the heart in the teleoroentgenogram (23). This change is reversible, and the heart size returns to normal after operative removal of the fistula. We have attempted to correlate the change in transverse diameter of the heart following operation with the degree of increase in cardiac output. The correlation coefficient between the variables is 0.12 which indicates a poor correlation.

The effect of an arteriovenous fistula upon blood volume has been discussed in an accompanying paper (21). Although there is no direct correlation between the increase in blood volume and the increase in cardiac output, there is, however, an indication that the blood volume tends to be increased in those patients with an elevated cardiac output and with clinical evidences of a large fistula. The effects of temporary occlusion of the fistula upon pulse rate, stroke volume, and cardiac output are likewise discussed separately (20).

From these studies it appears that many, but not all, patients with an arteriovenous fistula have a distinct elevation of the cardiac output above their normal level. It also appears that soon after the removal of the fistula there is a return of the output of the heart to an approximately normal value.

A complete explanation of the increased cardiac output in patients with an arteriovenous fistula is not yet possible. The usual explanation stresses the importance of the filling pressure of the ventricles, *i.e.*, the pressure in the atria and the great veins. It has, however, been demonstrated in patients with arteriovenous fistula or with large areas of hyperemia (17) that a marked change in cardiac output can occur without the appearance of any change in atrial pressure. For this reason atrial pressure changes are not a significant aid in explaining the increased cardiac output.

The observation of a marked fall in peripheral resistance when the fistula is open and indication from the ballistocardiogram that upon opening and closing the fistula the changes in heart rate and stroke volume appear within the first few beats suggest that the changed cardiac output depends on some property of the peripheral vascular bed. This view is discussed more fully elsewhere (17, 20).

Although our studies did not reveal specific data on cardiac failure in patients with arteriovenous fistula, they indicated a similarity of this condition to that of patent ductus arteriosus, severe anemia, and thyrotoxicosis in which cardiac failure occurs when the output is distinctly above normal. Therefore, in patients with arteriovenous fistula, a low output of the heart would not necessarily indicate circulatory inadequacy. On the other hand, the resistance of the cardiovascular system to stress varies so greatly in individuals that cardiac failure would not necessarily develop in those patients with the highest output.

As Starr and Jonas (24) had pointed out regarding patients with thyrotoxicosis, it is possible that a normal cardiac output in the face of the increased demands would be suggestive of cardiac failure. The combination of a normal cardiac output and a large fistula might be more indicative of cardiac insufficiency than merely a large cardiac output would be. This may be another reason why the cardiac enlargement or the elevated blood volume cannot be directly correlated with the increase in cardiac output.

SUMMARY AND CONCLUSIONS

A study of the cardiac output in 47 patients with arteriovenous fistulas was made by means of the low frequency critically damped ballistocardiograph before and after operative removal of the fistula. In 22 (47 per cent) of the patients there was a change of less than 25 per cent in the cardiac output in the presence of a fistula, when compared with the later normal values obtained for each patient after operative removal of the fistula. This change was not considered a significant one.

In 25 (53 per cent) of the patients with the fistula intact, there was elevation of the cardiac output of from 25 to 127 per cent above the postoperative (normal) level. Although, in general, these patients were the ones with clinical evidences of large fistulas, there was no striking correlation with the change in cardiac size, blood volume, or pulse rate, nor with duration of the fistula.

The evidence at hand indicates that the increased cardiac output is not due to an increase in filling pressure presented to the right side of the heart, but rather is related to change in the arterial tree.

Misses Maurine Giese and Margaret Steele gave valuable technical assistance.

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THE EFFECT OF TEMPORARY OCCLUSION OF ARTERIOVENOUS FISTULAS ON HEART RATE, STROKE VOLUME, AND CARDIAC OUTPUT [1]

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In patients with an arteriovenous fistula temporary occlusion of the fistula results in several alterations in the circulation. The most commonly recognized of these is the slowing of the heart rate. This response, described originally in 1875 by Nicoladoni (1), was reported independently in 1890 by Branham (2). It is now almost universally referred to as Branham's sign. Subsequently, additional changes in arterial pressure (3), cardiac output (4, 5) and peripheral blood flow (6) have been noted by other authors. In the course of our studies on the cardiovascular dynamics in patients with arteriovenous fistulas we have made further observations on Branham's sign and related phenomena. These data are the subject of the present report.

METHODS

The critically damped ballistocardiograph described by Nickerson and Curtis (7) was used in the determination of heart rate, stroke volume, and cardiac output. The method of its use is described in a separate report (5). Only patients with arteriovenous fistulas of the extremities were studied. The fistula was obliterated temporarily by rapid inflation of a blood pressure cuff to 200 mm. mercury pressure from a large air reservoir. The cuff was placed around the extremity in such a way that deflation did not affect the venous blood flow. This was done several times before taking the ballistic records in order to familiarize the patient with the procedure and to ascertain if there was discomfort upon application of pressure in the cuff. Ballistic tracings and arterial blood pressure measurement (determined by the auscultatory method) were obtained with the fistula both open and occluded. At least two sets of observations were made on each patient. In some instances 2 mgm. of atropine sulfate were injected intravenously, and the observations were repeated at the height of the atropine effect. Additional studies were made following operative removal of the arteriovenous fistula.

RESULTS

Twenty-five patients with arteriovenous fistulas resulting from trauma were studied. The studies were restricted to those patients with fistulas of the extremities which could be occluded by inflation of a blood pressure cuff. None of the patients had clinical evidence of cardiac failure, or any disease which might alter the responses of the cardiovascular system. All of the patients reported here have been included in a previously reported study on the cardiac output in patients with arteriovenous fistulas (5).

Observations on the effect of sudden occlusion of an arteriovenous fistula are recorded in Table I and shown graphically in Figure 1. In 17 pa-

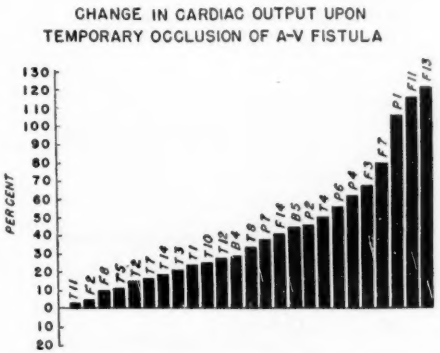

FIG. 1. CHANGES IN CARDIAC OUTPUT UPON TEMPORARY OCCLUSION OF A–V FISTULA

The cardiac output with the fistula open is expressed in per cent deviation from the cardiac output with the fistula occluded.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine, Atlanta, Georgia.

TABLE I

Summary of observations on 25 patients with arteriovenous fistulas of the extremities

Location of fistula and number of patients in group	Duration of fistula	Time of observation	Comment	Cardiac index	Stroke volume	Pulse rate	Arterial pressure
	months	*days before and after operation*		*liters per min. per sq. m.*	*cc.*	*beats per min.*	*mm. Hg*
Brachial (2)	4	4 before	Cuff off	4.8	126.5	70.4	128/79
		5 before	Cuff on	3.4	115.5	54	125/80
		9 after		4.0	105.9	68.8	122/66
Femoral (7)	4.1	6 before	Cuff off	5.7	132.8	77.4	119/90
		4 before	Cuff on	3.6	104.9	61.4	118/73
		15 after		3.5	93.0	69.8	115/74
Popliteal (5)	9.6	9 before	Cuff off	4.9	135.4	65.9	118/57
		11 before	Cuff on	3.7	102.5	55.8	117/81
		17 after		4.8	103.8	65.6	115/48
Tibial (11)	8.2	6 before	Cuff off	4.0	107.5	67.8	122/75
		7 before	Cuff on	3.2	91.9	63.4	124/79
		23 after		3.6	95.5	68.4	114/66

tients (68 per cent) the heart rate decreased more than 4 beats per minute and in one case as much as 32 beats per minute. In the remaining eight cases the rate changed only 4 or less beats per minute. In 19 patients (76 per cent) stroke volume decreased 10 cc. or more per stroke. The effects on the cardiac index (cardiac output in liters per minute per square meter of body surface) varied from no change to a decrease of 3.6 liters. In 22 patients (88 per cent) it decreased 0.5 liters

TABLE II

Observation of the effect of temporary occlusion of an arteriovenous fistula before and after administration of atropine

Location of fistula patient	Comment	Cardiac output	Cardiac index	Stroke volume	Heart rate	Arterial pressure
		liters per min.	*liters per min. per sq. m.*	*cc.*	*beats per min.*	*mm. Hg*
Femoral (7)	Cuff off	10.3	5.8	124	83	110/60
	Cuff on	6.5	3.7	107	61	115/70
	Atropine—2 mgm. intravenously					
	Cuff off	12.3	7.0	123	100	125/85
	Cuff on	8.6	4.9	83	104	145/95
Femoral (13)	Cuff off	12.0	6.5	158	76	120/55
	Cuff on	6.1	3.3	139	44	125/70
	Atropine—2 mgm. intravenously					
	Cuff off	9.7	5.3	105	93	130/85
	Cuff on	7.9	4.3	84	94	150/90
Popliteal (4)	Cuff off	11.6	6.0	140	83	115/60
	Cuff on	6.8	3.5	110	62	115/70
	Atropine—2 mgm. intravenously					
	Cuff off	15.6	8.0	127	123	135/90
	Cuff on	12.1	6.2	102	119	140/100
Popliteal (6)	Cuff off	5.8	3.6	93	62	110/60
	Cuff on	4.2	2.6	70	59	115/75
	Atropine—2 mgm. intravenously					
[illegible]	Cuff off	8.8	5.5	86	102	120/65
[illegible]	Cuff on	6.4	4.0	62	102	130/85
Tibial (4)	Cuff off	9.4	5.0	132	71	120/60
	Cuff on	6.9	3.6	120	57	120/80
	Atropine—2 mgm. intravenously					
	Cuff off	9.8	5.2	100	98	140/90
	Cuff on	5.6	3.0	59	96	160/100

or more, which, under the conditions of the study, probably represents a minimal detectable change. Changes in arterial pressure were not large, but almost uniformly consisted of an increase in diastolic pressure and frequently an increased systolic pressure upon occlusion of the fistula.

Additional studies on the effect of compression of the fistula were made on five patients who had received intravenously 2 mgm. of atropine sulfate (Table II). In all the atropine produced an increased pulse rate and in some an increase in cardiac index above the basal level. Upon inflation of the cuff the change in pulse rate was not greater than 4 beats per minute. Despite this relatively small change in heart rate, the decrease in stroke volume varied from 21 to 41 cc. and in cardiac output from 1.8 to 4.2 liters.

DISCUSSION

The advantages of the ballistic method for determination of relatively rapid changes in cardiovascular dynamics have been enumerated previously (5, 8). One disadvantage in the use of the ballistocardiograph is that manual compression of the fistula interferes with the movements of the ballistic bed. Therefore, a blood pressure cuff was used. This means of temporarily occluding the fistula occludes not only the fistula but also all of the circulation to the extremity distal to the cuff.

Studies on normal subjects, and on normal extremities of patients with arteriovenous fistula elsewhere in the body, have shown that rapid inflation of the cuff to 200 mm. mercury pressure produced practically no change in cardiac output or pulse rate. In one patient, in whom the fistula was also occluded with a Matas compressor (compression of only the major vessel entering the fistula) (9), it was found that the cuff and compressor produced identical changes in heart rate and cardiac output.

The findings reported here confirm Branham's observations. The rapidity of the reaction suggests that a nervous reflex is involved. Lewis and Drury (6) observed that they could block the reaction by the preliminary administration of atropine. Rieder (10) was unable to confirm this, but it is likely that his dosage of atropine was inadequate. The present results confirm the findings of Ellis and Weiss (11), and of Kramer and Kahn (12), that atropinization can block the change in pulse rate. This effect of atropine was interpreted as evidence that the pulse slowing response is mediated by a nervous mechanism, and by the vagus nerve in particular.

The specific stimulus which activates the bradycardiac response is not completely understood. It has been suggested that the vascular bed in and around the fistula may take on a function similar to the carotid sinus or aortic depressor areas in that local pressure changes are reflected in reflex changes in cardiac function. This does not appear to be the case. Observations (13, 14) have revealed that the pulse rate changes occur in patients with high spinal anesthesia. In these patients the area of the fistula is probably devoid of functional nervous connections, but it is not certain that complete denervation is present.

Several other systemic circulatory alterations take place when an arteriovenous fistula is occluded. Measurements of the pressure in the right atrium have failed to show any alteration, whereas the arterial pressure is found to rise. The latter phenomenon offers the most likely explanation for the slowing of the pulse rate. It is an established physiologic principle (Marey's law) that the pulse slows when the blood pressure rises. The origin of this type of reflex response appears to occur in the pressure sensitive end organs in the carotid sinus and aortic arch. In addition, experience in normal human subjects has shown that drugs which cause an increased arterial pressure with little change in cardiac output, such as paredrine, usually cause a slowing in the heart rate (15).

The present observations also demonstrate a striking decrease in stroke volume upon compression of the fistula. As a result of the summation of changes in heart rate and stroke volume the cardiac output is reduced. However, the reduction in stroke volume appears to be independent of the pulse rate change. Atropine, which abolishes the heart rate change, does not prevent the reduction in stroke volume. In fact, in the cases observed, the stroke volume reduction in occlusion of the fistula is greater with atropine than without. This effect is probably the result of the shorter filling time which is a consequence of the higher heart rate during atropinization.

The nature of the mechanism responsible for the change in stroke volume upon opening or

closing the fistula is of considerable interest. The change occurs rapidly, significant alterations appearing within the first few heart beats. Theoretically it appears that the stroke volume may be altered by one of three mechanisms: changes in the pressure gradient filling the ventricles, variation in the completeness of systolic emptying of the ventricles, and variation in the degree of diastolic relaxation of the ventricles.

Studies using the method of right heart catheterization have revealed evidence regarding the first possibility. It has been demonstrated (16) that there is no change in the mean right atrial pressure when the fistula is opened and closed, although there may be large changes in stroke volume. Changes in atrial pressures do not then appear to be the cause of the increased stroke volume. Nevertheless, one qualification remains in that a pressure gradient cannot be determined by measuring the pressure at a single point in the system. Pressure in the ventricle may change, hence the gradient filling the ventricle would be altered.

There is, however, no direct evidence regarding the completeness of systolic emptying and the changes in diastolic relaxation of the ventricles in normal man. In addition to invoking the passive increase of fluid movement on opening the fistula as an explanation of the increased stroke volume there is the possibility of involvement of some nervous mechanism. Such a mechanism may influence the degree of relaxation of the ventricles, and the necessary stimulus could originate in the venous bed. If a nervous reaction is involved, it must occur rapidly and not be blocked by atropine.

Changes in cardiac output and stroke volume similar to these reported here may be observed in normal subjects by the production of large areas of reactive hyperemia (16). If blood pressure cuffs are placed around the upper thighs and inflated to 200 mm. mercury pressure, little change in heart rate and cardiac output is produced. If the tourniquets are kept on for 20 minutes, and observations are made at the time of release, striking changes in heart rate and output may be noted. The heart rate increases, and the cardiac output per minute almost doubles. These changes, as those in our patients with arteriovenous fistulas, occurred without significant alterations in atrial pressure.

After operative removal of the fistula the cardiac output was found to be nearly the same as that observed previously upon temporary compression of the fistula. Cardiac output is such a variable function, changing markedly with even emotional factors, that it is difficult to say whether the failure of the values to coincide exactly always represents a real difference in output as related to the length of time that the fistula is closed, or whether it represents variations in the conditions under which the values were obtained.

SUMMARY AND CONCLUSIONS

The changes in heart rate and output occurring upon temporary compression of an arteriovenous fistula have been studied in a group of 25 patients.

In 68 per cent of the patients there was a significant decrease in heart rate (Branham's sign) which occurred almost immediately upon compression of the fistula and which was blocked by use of atropine.

In 88 per cent of the patients there was a decrease in stroke volume and cardiac output which occurred rapidly and simultaneously, but which was not altered by the use of atropine.

The evidence indicates that the change in heart rate results from a nervous reflex mediated by the vagus nerve, and most likely arises from alterations in arterial pressure. It appears that the change in stroke volume is due to variations in diastolic relaxation of the ventricle or to variation in the completeness of systolic emptying.

Although exactly comparable observations are difficult to obtain, the cardiac output found immediately upon closure of an arteriovenous fistula is approximately the same as that found several weeks after operative removal of the fistula.

Misses Mary Margaret Steele and Maurine Giese gave valuable technical assistance.

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THE BLOOD VOLUME IN PATIENTS WITH ARTERIOVENOUS FISTULAS [1]

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There has been much controversy concerning the changes in blood volume which occur in the presence of arteriovenous fistulas. The large number of vascular injuries treated at Ashford General Hospital during the recent war offered an unusual opportunity to study this problem. This paper reports observations on the blood volume changes in a group of 41 such patients studied before and after operative removal of an arteriovenous fistula.

In 1924 Holman (1) reported his studies on animals with arteriovenous fistulas. He used the brilliant vital red method of blood volume determination, obtaining a single dyed specimen four minutes after the injection of the dye. He found that the blood volume increased in the presence of a fistula. Later he reported similar blood volume studies in patients with arteriovenous fistulas (2, 3); in most instances there was a reduction in blood volume following removal of the fistula. In their studies on blood volume Rowntree and Brown (4) included seven patients with arteriovenous fistulas, three of whom had congenital lesions. Although the average blood volume was slightly above the normal value, in only one patient was there a distinctly increased blood volume. Pemberton and Saint (5) reported finding a slightly elevated value in a single patient with a congenital communication between artery and vein. Reid and McGuire (6) expressed doubt regarding the blood volume changes in view of their findings on three patients studied before and after operation. In one patient there was essentially no change in blood volume; in another, a postoperative decline of 600 cc.; and in the other, a postoperative drop of 1,000 cc. and nine months later, a return to the preoperative volume. In two experimental animals they found that a slight increase in blood volume accompanied the fistula. Using the blue dye T-1824 in a single case, Ellis and Weiss (7) found the blood volume within normal limits, but they did not repeat the measurement postoperatively. Kennedy and Burwell (8) reported a large value in a patient with multiple communications between artery and vein. The volume decreased after operation, but later fluctuated considerably as the fistula recurred.

There are many theoretical and practical objections to the methods used in the earlier studies and more recent observations have been inadequate in number. Therefore, the studies reported here were made.

METHODS

All determinations reported here were made on patients under basal conditions, at least 12 hours after the last meal and before arising in the morning. The plasma volume was measured using the blue dye T-1824 recommended by Gregersen, Gibson, and Stead (9). All blood samples were taken from the antecubital veins, using a dry syringe, and avoiding the hemoconcentrating effect of a tourniquet. After obtaining a dye-free sample, 3 cc. of an 0.1 per cent solution of the dye was injected from a calibrated syringe, and the syringe was rinsed three times with blood. Care was taken to prevent and note any extravascular loss of the dye. Exactly 10 minutes after the dye injection, a dyed sample of blood was obtained, again with precautions against hemolysis and hemoconcentration. In some patients, but not all, an inlying needle was left in place and serial samples obtained in order to determine the rate of disappearance of the blue dye.

The blood samples were placed in small tubes under a layer of mineral oil, allowed to clot and retract, then were centrifuged gently. The samples were discarded if there was evidence of lipemia or hemolysis. A Coleman Junior Spectrophotometer or a Nickerson decade photometer (10) was used in determining the concentration of

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine, Atlanta, Georgia.

the dye T-1824. This was done by measuring the light absorption of the dyed serum samples relative to the dye-free sample at a wave length of 620 millimicrons. Previously the apparatus had been calibrated with known amounts of dye in serum.

Blood specimens were also obtained for hematocrit reading, hemoglobin, and total protein determinations. Heparin was used as the anticoagulant in the hematocrit tubes. The hemoglobin concentration was determined by measuring the optical density of a dilute alkaline solution of blood. Total protein was determined by the density method of Barbour and Hamilton (11).

The plasma volume was calculated from either the dye concentration value obtained from extrapolation of the disappearance curve plotted semilogarithmically (12), or from the single 10-minute point as suggested by Gregersen (13). The total blood volume was determined from the plasma volume and the hematocrit reading. To facilitate comparison between patients of different size, the blood volume per square meter of body surface was calculated.

Additional studies, reported in detail elsewhere (14, 15), were made on many of these patients. Included were roentgenographic estimations of the heart size (obtained by measuring the transverse diameter of the heart on a teleoroentgenogram), and cardiac output determinations (by the ballistic method).

RESULTS

Observations were made on 41 patients, and the essential findings are reported in Table I. All patients had arteriovenous fistulas resulting from trauma. As far as could be determined, all the patients were healthy young adults before injury, and none had complicating injuries or illness that might have altered the blood volume. There was one woman in the group. Table I shows that the location of the lesions varied considerably. In all patients there were characteristic physical signs of the fistula. The duration between removal of the fistula and the infliction of the wound which caused it varied from slightly more than two months to two years. In no patient was there evidence of frank cardiac failure. The operative removal of the fistula was done with either sodium pentothal or continuous spinal anesthesia. In all instances the fistula was successfully removed, and the postoperative course was uneventful. With the exception of those determinations done on the third postoperative day, the patients were usually allowed out of bed by the time the blood volume determinations were made.

In the entire group, before operation the blood volume per square meter of body surface ranged from 2,400 cc. to 4,030 cc. Ten days or more after operation it ranged from 2,330 cc. to 3,380 cc. In 23 patients (56 per cent) the change was less than 200 cc. A change of 200 cc. or less was not considered a significant change.

TABLE I

Summary of observations on 41 patients with arteriovenous fistulas

Location of fistula and number of patients in group	Duration of fistula	Time of observation	Plasma volume	Total blood volume	Deviation from normal blood volume	Hematocrit reading	Total plasma protein	Heart size
	months	*days before and after operation*	*cc.*	*cc.*	*cc. per sq. m.*		*gms. per 100 cc.*	*cm.*
Carotid (*1*)	3.5	1 before	2650	5100		48.2	6.7	−1.5
		21 after	2420	4385	−440	44.8	6.1	
Subclavian (*4*)	3.5	7 before	2848	5540	−168	49.1	6.0	+0.5
		12 after	2932	5387		46.1	6.4	
Axillary (*5*)	8.1	12 before	2990	5700	−122	47.7	6.3	−5.6
		20 after	2806	4947		46.8	6.4	
Brachial (*3*)	3.5	6 before	2620	5053	90	47.8	6.4	0
		10 after	2970	5220		49.4	6.5	
Iliac (*4*)	6	14 before	3146	5616	−365	44.3	6.9	−0.6
		14 after	2667	4957		46.4	6.3	
Femoral (*13*)	6.1	6 before	2972	5645	−238	47.6	6.2	−1.2
		14 after	2618	5169		49.4	6.4	
Popliteal (*5*)	6.8	7 before	2827	5584	− 76	49.2	6.4	−0.2
		15 after	2856	5476		47.7	6.8	
Tibial (*6*)	8.7	3 before	2735	5068	−116	45.6	6.2	−0.4
		19 after	2661	4865		45.0	6.5	

In 18 patients (44 per cent), after removal of the fistula there was a decrease in blood volume from 200 cc. to 1,060 cc. per square meter body surface. In many instances these changes were confirmed by repeated observations. On the other hand, in none of the patients was there a significant increase in blood volume following operation.

The hematocrit readings varied slightly, most often showing a tendency to fall somewhat after operation. Therefore, the calculated red blood cell volume underwent similar changes. The hemoglobin and total protein concentration did not undergo marked alteration. The cardiac output data and changes in heart size have been described in detail elsewhere (14).

There has been wide use of the blue dye T-1824 (9) in the determination of plasma volume. In the present studies the disappearance of the dye was plotted semilogarithmically as recommended by Gregersen and Rawson (12). More recently Gregersen (13) has suggested that in certain situations a single dyed specimen obtained 10 minutes after dye injection is almost as reliable as an extrapolated value for dye concentration obtained from the disappearance curve of the dye. He found this true in normal patients and patients in shock. In the case of many patients reported here there were obtained both 10-minute samples and multiple specimens for plotting the disappearance slope of the dye. There was no evidence that the disappearance rate of the dye was affected by the presence of the arteriovenous fistula, and the plasma volume as calculated by the two methods did not differ significantly. It must be remembered, however, that none of these patients had frank congestive heart failure. It is possible that the prolonged mixing time of cardiac failure might invalidate the 10-minute point method. Because of the limited time that the patients were available, and because multiple determinations were made on a large group, the 10-minute point method was used extensively.

The calculation of the total blood volume from the hematocrit reading and the plasma has been subject to criticism (16, 17). This is primarily because the peripheral venous hematocrit reading is imperfect from the standpoint of indicating the proportion of cells in the blood in flow, and because the plasma trapped among the cells in centrifugation prevents arrival at a true cell-plasma ratio by hematocrit reading. Despite the handicaps, a value determined by this means may serve many useful purposes. It appears particularly useful in measurements, such as these reported here, where comparative observations are made on the same person who has minimal hematocrit changes.

Because it appears to be at the expense of the ratio of cells to plasma that the body maintains the total blood volume at a constant level, we found the total blood volume the most stable and useful value for these studies. Since the body can mobilize fluid and protein with relative rapidity, the plasma volume appears to vary to compensate for changes in the red cell volume. Studies on the effect of simple hemorrhage show that these changes in plasma volume first occur with a slow readjustment of the red cell volume and hematocrit reading to the normal value (18). Similar changes may be seen soon after an operation, with its accompanying blood loss and dehydration. Because of this, the plasma volume is subject to much more fluctuation than is the total blood volume, and knowledge concerning it was of less value in these studies than was evidence of changes in the total blood volume. Although some of the postoperative measurements reported here were made relatively early following operation, an effort was made to delay them long enough to avoid any immediate effects of the operation. Only observations made 10 days or more after operation were used in the calculation of the normal values. Almost all of the patients were ambulatory by this time. The values obtained fell in the same range as those reported previously for normal male subjects, the average blood volume being near 2,800 cc. per square meter of body surface (19).

The results of these studies are expressed graphically in Figure 1, considering the postoperative blood volume as the normal for each patient. A majority of the patients showed a change of less than 200 cc. in blood volume per square meter body surface. This degree of change (about 7 per cent of the normal value) may be expected independently, without direct effect on the arteriovenous fistula. Such factors as technical errors in blood volume determination, changes resulting from operation, and bed rest could readily account for a 7 per cent variation. On the other hand, following operation, although no patient had an in-

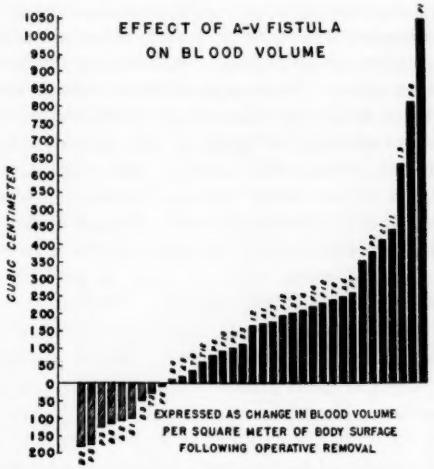

FIG. 1. EFFECTS OF AN A–V FISTULA ON BLOOD VOLUME
The change in blood volume from before operation to the postoperative period is expressed in cubic centimeters per square meter of body surface.

crease in blood volume greater than 200 cc., 18 patients (44 per cent) had a decrease of more than 200 cc. Insofar as could be determined, this decrease was not related to any technical errors or to any incident of the operation or convalescence not directly related to the removal of the arteriovenous fistula. In several instances repeated observations before and after operation verified the presence of the change, and revealed that the decline was in progress by three days postoperatively, and was apparently complete by 10 days. Observations were not made earlier than three days postoperatively because it was felt that changes incident to the operation might cause alterations in blood volume not due to the actual removal of the fistula.

The findings reported here are in agreement with earlier reports that blood volume may be significantly elevated in patients with arteriovenous fistulas. It is not a consistent reaction, however, as changes were noted in only less than half of the patients studied. In an attempt to elucidate the cause of this blood volume change, an effort was made to relate the blood volume change with other alterations in the circulation.

It appears that the size of the fistula is a major factor in determining the increase in blood volume. For instance, two of the four patients with the greatest change in blood volume had fistulas involving the iliac vessels. However, the actual size of a fistula is difficult to determine. Clinical evidences, such as the size of the vessels involved and the intensity of the thrill or murmur, give some evidence, although not entirely accurate, regarding the magnitude of the shunt. Actual measurement of the fistulous opening upon operation has not proved successful because of the variable de-

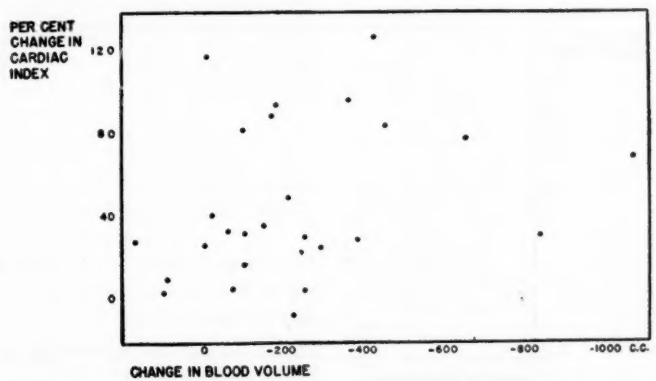

FIG. 2. A CHART DEMONSTRATING THE RELATIONSHIP BETWEEN THE CHANGE IN CARDIAC OUTPUT AND THE CHANGE IN BLOOD VOLUME FOLLOWING REMOVAL OF AN A–V FISTULA
The change in blood volume is expressed in cubic centimeters per square meter of body surface.

gree of vascular spasm. Measurement of the opening after removal from the body is difficult for the same reason plus the shrinking effect of tissue fixation.

In many patients with arteriovenous fistulas the resting level of cardiac output is above normal (14). Since the elevation of the cardiac output above normal might be proportional to the size of the fistula, correlation of the increase in blood volume with the increase in cardiac output was attempted (Figure 2). To avoid the considerable range in basal cardiac output from person to person, the blood volume change was plotted against the per cent of deviation from normal of the cardiac output in the particular patient observed. In other words, if the preoperative cardiac output was twice that found as the postoperative basal value, a 100 per cent increase in cardiac output was plotted. The correlation coefficient was 0.26, a value which indicated that the correlation was not good. It appears, however, that the size of the fistula may be at least one of several factors in the changes leading to an increased blood volume.

It has repeatedly been observed that in patients with arteriovenous fistulas the heart size, determined roentgenologically, may become abnormally large, and after the fistula is removed, it returns to normal size (20). Since there is evidence to suggest that the heart size is related to the blood volume, these two findings were correlated. Twenty-nine of the patients had satisfactory teleoroentgenograms of the heart made before and after operation. In many instances there were repeated observations. The change of blood volume was plotted against the change in the transverse diameter of the heart in Figure 3. The correlation coefficient of value 0.36 indicates that a significant degree of correlation was not present. This is not entirely surprising in view of recent evidence that in normal subjects the rapid increase in blood volume of about 1,000 cc. failed to produce a distinct change in heart size (21). Holman found a change in heart size in dogs upon increasing or decreasing the blood volume, but he studied changes of much greater magnitude than seen in patients with arteriovenous fistulas (1). A change of 500 cc. in a dog such as he studied is equivalent to a change of approximately 4,000 cc. in a human. In neither of the afore-mentioned studies was the increase in blood volume accompanied by an increase in extracellular fluid volume, which if present might alter the situation considerably. It appears likely that other factors, such as the increased demand on the heart, play a major role.

An attempt was made to relate the increase in blood volume to the duration of the fistula. Our data are not satisfactory for this purpose, however, because so few patients had large fistulas of long duration. Most of the patients with lesions of con-

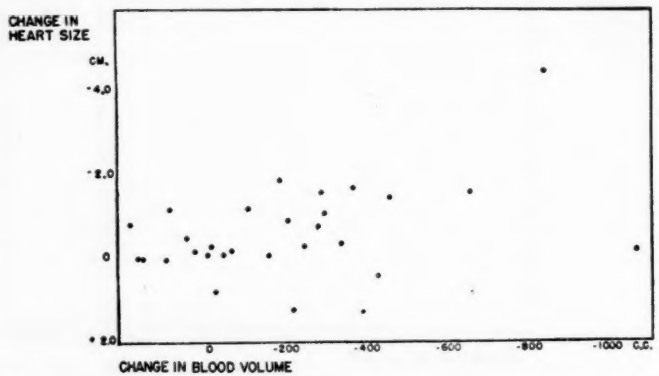

FIG. 3. THE CHANGE IN BLOOD VOLUME PLOTTED AGAINST THE CHANGE IN HEART SIZE

The change in heart size is expressed as the change in transverse diameter of the heart determined from the teleoroentgenogram.

siderable age had rather small fistulas which had often escaped notice by the patient and physician.

Our results demonstrate that in some patients with arteriovenous fistulas there is a significant increase in blood volume. After operative removal of the fistula, the blood volume, if elevated, returns to normal. Since the basic factors controlling blood volume in the normal person are so little understood, it is not surprising that we cannot explain the increased blood volume in these patients.

It is possible that with some large venous channels engorged with blood, the mechanisms controlling the blood volume might be misled. Since the amount of blood remaining in the rest of the vascular bed would be less than normal, increased production of blood might be stimulated. This could be the basic mechanism causing the increased blood volume. There is also the possibility that the increased venous pressure in the region of the fistula may dilate the vascular bed in that region, thus increasing its capacity for blood and spurring the demand for more blood.

It has been suggested that the increase in blood volume seen in patients with arteriovenous fistulas merely represents the increase in blood volume seen in patients with any type of congestive heart failure (6); that is, it may be the increase in blood volume of cardiac failure rather than that of arteriovenous fistula *per se*. Although none of the patients observed in these studies developed the complete clinical picture of cardiac failure, it cannot be denied that there may be a similar mechanism in the causation of their increased blood volume.

SUMMARY AND CONCLUSIONS

1. Observations on the blood volume have been made before and after operative removal of an arteriovenous fistula in 41 patients.

2. In 56 per cent of the patients there were changes in blood volume of less than 200 cc. per square meter of body surface. This was not considered a significant variation.

3. In 44 per cent of the patients the decrease in blood volume after operation ranged from 200 cc. to 1,060 cc. per square meter of body surface, indicating an abnormally large blood volume in the presence of the fistula.

4. The relationship of the change in blood volume to the size and duration of the fistula, the cardiac output, and the changes in heart size have been studied. In general, the patients with an elevated blood volume were those with clinical evidences of a functionally large fistula.

Miss Maurine Giese and Miss Mary Margaret Steele gave valuable technical assistance.

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THE EFFECT OF ADRENOCORTICOTROPHIC HORMONE ON CHILDREN WITH THE NEPHROTIC SYNDROME. II. PHYSIOLOGIC OBSERVATIONS ON DISCRETE KIDNEY FUNCTIONS AND PLASMA VOLUME[1]

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INTRODUCTION

Inability to predict the time of occurrence of spontaneous diureses in the nephrotic syndrome has retarded investigations of the physiologic changes which may precede, accompany, and follow these heartening but enigmatic events. It has been demonstrated recently that several agents (1–5) are capable of *inducing* diureses at a predictable time in a far higher percentage of trials than could be attributed to chance. Adrenocorticotrophic hormone (ACTH) is one such agent, and the abrupt, profuse, and sustained diureses which may accompany or follow its administration are clinically similar to the dramatic diureses which occur spontaneously. Clinical observations on the effect of ACTH[4] on a group of children with the nephrotic syndrome is the subject of a separate report (6) in which the possible clinical significance of the results is also discussed. Serial physiologic measurements were made on selected patients before, during, and after administration of ACTH. These observations were directed toward an increased understanding of the syndrome itself, and especially of the mechanism of diuresis rather than toward evaluation of possible relationships between the disease and pituitary or adrenocortical function. As an integral part of the approach, an attempt was made to disclose, if possible, consistent changes which might precede the outset of diuresis and, to this end, as many relevant physiologic measurements as practicable were made. These included: blood volume; discrete kidney functions; concentrations of electrolytes and nitrogenous constituents of serum; metabolic balances of electrolytes and nitrogen; serum lipid fractions; and urinary protein and sediment.

It has not been possible from any one or combination of changes to predict when diuresis would occur in relation to administration of ACTH. The most striking changes observed after the outset of diuresis were increases in plasma volume and unexpectedly large increases in glomerular filtration rate (GFR). These changes together with observations on other discrete kidney functions are described in the present paper. Observations on other measurements will be the subject of a subsequent report (7).

SUBJECTS AND METHODS

Observations were made on eight children with the nephrotic syndrome whose clinical status and course during ACTH administration is described in a separate report (6). Clinical data relevant to the observations presented here are included in Table I.

Various combinations of the following measurements

[1] Presented in part before the Society of Pediatric Research at French Lick, Indiana, on May 9, 1950.

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[4] We wish to thank Dr. John R. Mote of the Armour Laboratories for allocating the ACTH used in these observations.

were made before, during, and after administration of ACTH: daily weight; plasma volume and hematocrit; clearances of inulin (C_{IN}); endogenous creatinine (C_{CR}); thiosulfate (C_{THIO}) and p-aminohippurate (C_{PAH}); and maximum tubular excretion of PAH ($T_{M_{PAH}}$). In three children (I. C., K. N., J. S.), 24-hour clearances of endogenous creatinine (C_{CR-24}) were measured. These children were on low salt (20 meq. per day) but otherwise normal diets. Most of the time they were ambulatory and normally active.

Methods for inulin, p-aminohippurate, urea (8) and endogenous creatinine (9), in blood and urine, and the catheterization and infusion technic used for short-term simultaneous clearances have been described (8). Serum and urine thiosulfate concentrations were determined by the method of Newman, Gilman, and Phillips (10). C_{CR-24} was calculated using 24-hour creatinine excretion and mid-point serum values interpolated from creatinine determinations made one to three days apart. Plasma volume was measured with T-1824 using a single 10-minute blood sample and the acetone extraction method of Chinard and Eder (11) for lipemic sera. Blood volume was calculated from plasma volume and hematocrit (12).

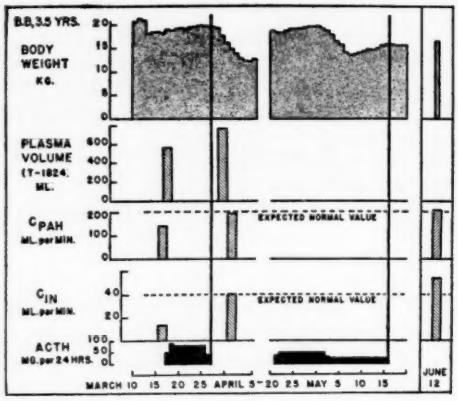
B.B.,3.5 YRS.
BODY WEIGHT KG.
PLASMA VOLUME (T-1824) ML.
C_{PAH} ML. per MIN.
EXPECTED NORMAL VALUE
C_{IN} ML. per MIN.
EXPECTED NORMAL VALUE
ACTH MG. per 24 HRS.
MARCH 10 15 20 25 APRIL 5 10 20 25 MAY 5 10 15
JUNE 12

FIG. 1. RELATION OF CHANGES IN BODY WEIGHT, PLASMA VOLUME, AND CLEARANCES OF INULIN AND P-AMINOHIPPURATE TO ADMINISTRATION OF ACTH IN A CHILD WITH THE NEPHROTIC SYNDROME

RESULTS [5]

Changes observed in discrete kidney functions and plasma volume in relation to hormone administration are given in Table I. Data from B. B. and I. C. are shown graphically in Figures 1 and 2.

Glomerular filtration rate

The most striking changes observed during or following diureses associated with ACTH administration were marked increases in C_{IN}. Such increases were observed on eight occasions in six of the eight children and were greatest in patients with low initial values. Thus increases in C_{IN} of 179, 211, 190, 50, and 256% above control values were observed respectively in I. C., K. N., B. B., M. L., and S. S.[6] In B. B., this change in C_{IN} represents *an increase from 35 to 133% of normal* (Figure 1). In the three children whose initial values were within the normal range, measurements of C_{IN} showed no significant increase in two (J. S. and R. T.), but revealed an increase to the high value of 123% of normal in one (R. K.). Failure to observe an increase in C_{IN} following diuresis (as in I. C., February 1) may possibly be due to failure to make the measurement at the proper time as discussed later.

In an attempt to determine when changes in GFR occurred and to follow their course, 24-hour clearances of endogenous creatinine were measured. The ratio of $C_{CR} : C_{IN}$ is irregularly greater than one in children with kidney disease (17). Consequently, repeated short term measurements of this ratio were made to relate changes in the 24-hour clearance to changes in GFR. Even though changes in this ratio were taken into account, C_{CR-24} was interpreted only as reflecting changes in rather than as an absolute measure of GFR. Despite these reservations, changes in C_{CR-24} were sufficiently large to demonstrate that GFR increased during the first 24 hours of diuresis, as shown in Figure 2. Additional impressions concerning changes in GFR during and following ACTH administration are gained from inspection of the data from I. C.[7]

[5] The frequency with which diuresis has been observed with ACTH in children with the nephrotic syndrome is reported separately (6). In this group of eight children, selected because measurements had been made both before and after ACTH, a diuresis failed to occur in only one of 14 courses. This failure occurred when only 50 mg. per day for three and one half days was given to a child (I. C.) who diuresed on three other occasions with larger dosages.

[6] Only a small fraction of the observed increases in C_{IN} could be related to increases in rate of urine flow during diuresis.

[7] In this patient and in others receiving cortisone in larger dosages no diuresis occurred and no increase in GFR was observed. On the contrary, a marked fall in GFR was observed in S. S. 15 days after he had received 100 mg. of cortisone in seven days. There are not sufficient data to indicate the significance of this observation.

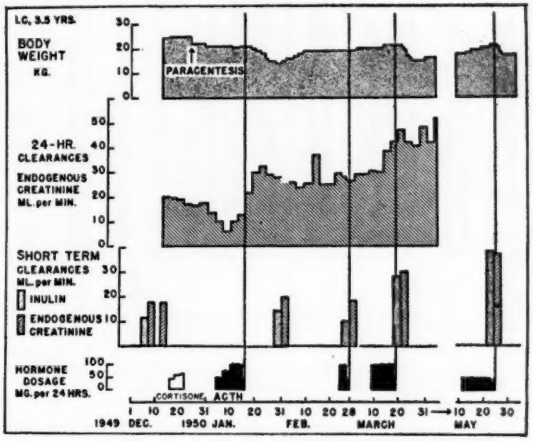
I.C, 3.5 YRS.
BODY WEIGHT KG.
PARACENTESIS
24-HR. CLEARANCES
ENDOGENOUS CREATININE ML. per MIN.
SHORT TERM CLEARANCES ML. per MIN.
INULIN
ENDOGENOUS CREATININE
HORMONE DOSAGE MG. per 24 HRS.
CORTISONE
ACTH
1 10 20 31 10 20 31 10 20 28 10 20 31 10 20 30
1949 DEC. 1950 JAN. FEB. MARCH MAY

FIG. 2. RELATION OF CHANGES IN BODY WEIGHT AND CLEARANCES OF INULIN AND ENDOGENOUS CREATININE TO ADMINISTRATION OF ACTH IN A CHILD WITH THE NEPHROTIC SYNDROME

The decrease in C_{CR-24} seen during the first course of ACTH administration (January 4 to 16) has been observed in other patients and was often associated with a marked rise in serum urea nitrogen and endogenous creatinine. These changes suggest a temporary fall in GFR during ACTH administration. The low value for C_{IN} measured on February 28, one day after a three day course of ACTH which did not induce diuresis, lends support to this impression.[8] The pattern of changes in C_{CR-24} in Figure 2 suggests that each diuresis was accompanied by an increase in GFR and followed by a decrease from the maximum value with subsequent stabilization at a value slightly or markedly above the control. It is possible that the fall in GFR may already have occurred when C_{IN} was measured on February 1, 1950, since re-accumulation of edema already had begun. Repeated responses to ACTH may, however, be associated, as shown in Figures 1 and 2, with a progressive increase in GFR to normal (I. C.) or even "supernormal" values (B. B.).

Changes of this order of magnitude in C_{IN} in children with diseased kidneys must raise the question of the validity of C_{IN} as a measure of GFR under these conditions. This question becomes especially pertinent in view of the consistent changes observed in three children (I. C., K. N., and B. B.) in the $C_{CR}:C_{IN}$ ratio which decreased from a range of 1.5 to 1.9 before to a range of 1.0 to 1.4 after diuresis. However, the reasonably good agreement between C_{IN} and C_{THIO} ($C_{THIO}:C_{IN}$ ratios ranging from 0.9 to 1.2) at both decreased and increased values of C_{IN} provides evidence that C_{IN} was a measure of GFR in these children. The changes observed in the $C_{CR}:C_{IN}$ ratio are unexplained at present.

Effective renal plasma flow (C_{PAH})

Significant and consistent increases in C_{PAH} were observed to accompany increases in C_{IN} during diureses occurring with ACTH. However, increases in C_{PAH} were consistently less than those in C_{IN} so that in the six children (I. C., K. N., B. B., R. K., M. L., and S. S.) who showed an increase in C_{IN} the $C_{IN}:C_{PAH}$ ratio increased from a range of 0.10 to 0.17 before to a range of 0.22 to 0.32 after diuresis. Whether these changes

[8] C_{CR}, measured simultaneously with C_{IN}, showed a comparable decrease at this time. Failure of C_{CR-24} to reflect this decrease may be partly explained by the inaccuracy of calculating clearances using mid-point values for serum creatinine concentrations interpolated from determinations made on fasting samples taken at intervals of several days (18). C_{CR-24} was consistently higher than C_{CR} which might be expected from the fact that the latter was always measured early in the day with the subject at rest and in a fasting state, whereas the former might be affected by such factors as eating and activity.

TABLE I—*Measurements of discrete kidney functions* and plasma volume in relation to hormone administration*

The New York Hospital

Subject	Date	Hormone administration	Weight	C_{IN}	C_{CR}	C_{THIO}	C_{PAH}	T_{MPAH}	C_{IN}/C_{PAH}	C_{CR}/C_{IN}	C_{THIO}/C_{IN}	C_{IN}/T_{MPAH}	Plasma volume	Blood volume	Hematocrit	Comment
			kg.	*ml. per min.*	*ml. per min.*	*ml. per min.*	*ml. per min.*	*mg. per min.*					*ml.*	*ml.*	%	
I. C.	12/7/49		23.4	11.9	18.0	12.0	77.9	—	0.15	1.5	1.0	—				Duration of disease—7 mos.
F. Age 3.5 yrs.	12/12		24.4	—	18.0	—	—	—	—	—	—	—				Constant edema since outset with no spontaneous diuresis.
Ht. 96.0 cm.	12/16–21	Cortisone 335 mg. (6d)	24.7–25.2	—	—	—	—	—	—	—	—	—				
Wt. 15.2 kg.‡	1/4/50		21.4	—	—	—	—	—	—	—	—	—				
S.A. 0.62M²	1/4–16	ACTH 1000 mg. (13d)†	21.4–20.9	—	—	—	—	—	—	—	—	—				
	1/19		20.3	—	—	—	—	—	—	—	—	—				‖Diuresis 15th day after start of therapy (1/19).
	1/30		14.2	—	—	—	—	—	—	—	—	—				Minimum weight following diuresis.
	2/1		14.6	14.3	19.9	12.8	—	—	—	1.4	0.9	—				Outset of reaccumulation of edema.
	2/16		19.6	—	—	—	—	—	—	—	—	—				Maximum weight.
	2/23		19.1	—	—	—	—	—	—	—	—	—				
	2/24–27	ACTH 350 mg. (3½d)	18.5–19.1	—	—	—	—	—	—	—	—	—				No diuresis.
	2/28		19.3	10.1	18.6	—	70.9	—	0.14	1.8	—	—				
	3/7		19.6	—	—	—	—	—	—	—	—	—	663	1074	40.0	
	3/8–17	ACTH 950 mg. (9½d)	19.6–21.4	—	—	—	—	—	—	—	—	—				
	3/21		20.9	28.2	30.4	—	119	—	0.24	1.1	—	—				Diuresis 13th day after start of therapy (3/21)
	3/22		20.0	—	—	—	—	—	—	—	—	—	853	1218	31.4	
	3/27		15.0	—	—	—	—	—	—	—	—	—				Minimum weight following diuresis.
	3/30		15.4	—	—	—	—	—	—	—	—	—				Outset of reaccumulation of edema.
	5/10		17.5	—	—	—	—	—	—	—	—	—				
	5/11–24	ACTH 687.5 mg. (14d)	17.2–20.7	—	—	—	—	—	—	—	—	—				
	5/24		20.7	38.2	37.5	33.2	106	—	0.36	1.0	—	—				
	5/26		20.1	—	—	—	—	—	—	—	—	—				Diuresis 15th day after start of therapy (5/26).
	11/16		17.5	—	—	—	—	—	—	—	—	—				In apparent clinical remission 12/1/50.
Expected normal value §				*44.1*			*225*		*0.20*							
K. N.	1/20/50		20.5	8.1	16.6	—	71.9	—	0.11	2.0	—	—				Duration of disease—10 mos.
M. Age 3 yrs.	1/20–27	ACTH 675 mg. (8d)	20.5–20.6	—	—	—	—	—	—	—	—	—				Constant edema since outset with no spontaneous diuresis.
Ht. 91.5 cm.	1/29		20.3	—	—	—	—	—	—	—	—	—				Diuresis 9th day after start of therapy (1/29).
Wt. 14.1 kg.	2/3		14.3	25.2	30.1	—	94.2	—	0.27	1.2	—	—				
S.A. 0.58M²	2/6		12.2	—	—	—	—	—	—	—	—	—				Minimum weight following diuresis.
	2/9		12.5	—	—	—	—	—	—	—	—	—				Outset of reaccumulation of edema.
	3/3		18.4	—	—	—	—	—	—	—	—	—	553	819	34.0	Maximum weight.
	3/4–13	ACTH 950 mg. (9½d)	18.3–17.4	—	—	—	—	—	—	—	—	—				
	3/10		19.7	—	—	—	—	—	—	—	—	—				Diuresis 6th day after start of therapy (3/10).
	3/11		19.1	—	—	—	—	—	—	—	—	—				
	3/13		17.4	—	—	—	—	—	—	—	—	—	645	904	30.0	
	3/18		12.4	—	—	—	—	—	—	—	—	—				Minimum weight following diuresis.
	3/21		12.9	—	—	—	—	—	—	—	—	—				Outset of reaccumulation.
	4/20		15.9	—	—	—	—	—	—	—	—	—				
	4/21		16.2	—	—	—	—	—	—	—	—	—				Maximum weight.
	4/21–5/1	ACTH 537.5 mg. (11d)	16.2–15.4	—	—	—	—	—	—	—	—	—				Diuresis 8th day after start of therapy (4/29).
	4/29		17.2	—	—	—	—	—	—	—	—	—				
	4/30		16.6	—	—	—	—	—	—	—	—	—				
	4/31		16.1	—	—	—	—	—	—	—	—	—				
	5/4		13.9	—	—	—	—	—	—	—	—	—				Minimum weight following diuresis.
	5/31		16.1	27.6	38.2	—	79.8	—	0.35	1.4	—	—				
	11/17		15.5	—	—	—	—	—	—	—	—	—				In apparent clinical remission 12/1/50.
Expected normal value				*41.2*			*211*		*0.20*							No manifest edema.

* Each value represents the average of three or more 10 to 20 minute clearance periods.

† Daily dosage of ACTH was given in four divided doses at six hour intervals.

‡ Ideal weight for height and age (13) was used for the estimation of surface area (14) from which expected normal values for renal plasma clearances were calculated.

§ These values were calculated as follows:

$$\text{Expected normal value} = \frac{\text{Surface area of child } (M^2)}{1.73M^2} \times \text{normal adult value corrected to } 1.73M^2.$$

Since there is no evidence that a sex difference in renal functions exists in children before puberty, a single normal adult value for each discrete function was used to calculate expected normal values for both boys and girls. These values were arbitrarily taken as the average of mean values for normal men and women (15). Per unit of surface area, values for discrete kidney functions for children of this age are expected to be within the normal adult range (16).

‖ Day on which weight loss of 0.2 kg. or greater was first observed and which was followed by continued loss of body weight.

TABLE I—*Continued*

Subject	Date	Hormone administration	Weight	C_{IN}	C_{CR}	C_{THIO}	C_{PAH}	T_{MPAH}	C_{IN}/C_{PAH}	C_{CR}/C_{IN}	C_{THIO}/C_{IN}	C_{IN}/T_{MPAH}	Plasma volume	Blood volume	Hematocrit	Comment
			kg.	*ml. per min.*	*ml. per min.*	*ml. per min.*	*ml. per min.*	*mg. per min.*					*ml.*	*ml.*	%	
B. B.	3/16/50		18.3	14.0	28.1	16.2	148	—	0.09	2.0	1.2	—		—		Duration of disease—7 mos.
F. Age 3.5 yrs.	3/17		19.0	—	—	—	—	—	—	—	—	—	615	814	25.6	Constant edema since outset with no spontaneous diuresis.
Ht. 90.0 cm.	3/17–26	ACTH 740 mg. (9d)	19.0–19.8	—	—	—	—	—	—	—	—	—				
Wt. 13.7 kg.	3/28		19.3	—	—	—	—	—	—	—	—	—				Diuresis 11th day after start of therapy (3/28).
S.A. 0.57M²	3/29		17.7	—	—	—	—	—	—	—	—	—	774			
	3/31		14.8	40.6	44.5	39.0	197	—	0.21	1.1	1.0	—				
	4/3		12.4	—	—	—	—	—	—	—	—	—				Minimum weight following diuresis.
	4/20		18.6	—	—	—	—	—	—	—	—	—				Maximum weight.
	4/21		18.4	—	—	—	—	—	—	—	—	—				
	4/21–5/15	ACTH 893.8 mg. (25d)	18.4–15.9	—	—	—	—	—	—	—	—	—				
	5/1		19.1	—	—	—	—	—	—	—	—	—				Diuresis 10th day after start of therapy (5/1).
	5/7		13.6	—	—	—	—	—	—	—	—	—				Minimum weight following therapy.
	5/9		14.1	—	—	—	—	—	—	—	—	—				In apparent clinical remission 5/9–10/1.
	6/12		16.5	54.0	55.7	—	211	—	0.26	1.0	—	—				Reaccumulation of edema 10/1/50.
	11/2		18.2	—	—	—	—	—	—	—	—	—				13 days ACTH therapy (525 mg.) resulted in diuresis.
Expected normal value				*40.5*			*207*		*0.20*							
J. S.	3/6/50		13.9	41.2	57.9	46.3	196	—	0.21	1.4	1.1	—				Duration of disease—2.5 mos.
M. Age 3.2 yrs.	4/11		15.3	45.8	58.6	—	196	—	0.23	1.3	—	—				One spontaneous diuresis 4 weeks after outset.
Ht. 95.0 cm.	4/12			—	—	—	—	—	—	—	—	—	678	1064	38.0	Minimal edema at start of therapy.
Wt. 15.0 kg.	4/14–20	ACTH 625 mg. (6½d)	15.6–15.9	—	—	—	—	—	—	—	—	—				
S.A. 0.61M²	4/21		15.7	—	—	—	—	—	—	—	—	—	750	1143	36.0	
	4/22		15.2	42.8	46.4	—	193	—	0.22	1.1	—	—				Diuresis 8th day after start of therapy (4/22).
	4/24		14.7	—	—	—	—	—	—	—	—	—				Minimum weight following diuresis.
	8/31		18.0	—	—	—	—	—	—	—	—	—				
Expected normal value				*45.5*			*221*		*0.20*							Moderate intermittent peripheral edema 12/1/50.
R. K.																
F. Age 3.9 yrs.	7/10/50		27.4	37.1	48.1	—	212	—	0.18	1.3	—	—				Duration of disease—11 mos.
Ht. 100 cm.	7/11		27.8	—	—	—	—	—	—	—	—	—	933	1510	40.0	One diuresis in March 1950 following paracentesis.
Wt. 16.1 kg.	7/12		27.6	—	—	—	—	—	—	—	—	—				Reaccumulation of edema 4 months later.
S.A. 0.64M²	7/12–22	ACTH 500 mg. (11d)	27.6–27.8	—	—	—	—	—	—	—	—	—				
	7/23		27.2	—	—	—	—	—	—	—	—	—				Diuresis 11th day after start of therapy (7/23).
	7/27		19.3	56.0	64.5	—	233	—	0.24	1.2	—	—	918	1463	39.0	
	8/1		17.2	—	—	—	—	—	—	—	—	—				Minimum weight following diuresis.
	11/26		21.8	—	—	—	—	—	—	—	—	—				Reaccumulation of edema and spontaneous diuresis in October and again in November.
Expected normal value				*45.5*			*233*		*0.20*							

TABLE I—*Continued*

Children's Hospital of Philadelphia

Subject	Date	Hormone administration	Weight	C_{IN}	C_{CR}	C_{THIO}	C_{PAH}	T_{MPAH}	C_{IN}/C_{PAH}	C_{CR}/C_{IN}	C_{THIO}/C_{IN}	C_{IN}/T_{MPAH}	Plasma volume	Blood volume	Hematocrit	Comment
			kg.	*ml. per min.*	*ml. per min.*	*ml. per min.*	*ml. per min.*	*mg per min.*					*ml.*	*ml.*	%	
R. T.	1/4/50		17.5	49.4	—	—	160	31.3	0.31	—	—	1.6				Duration of disease—4 mos.
M. Age 3 yrs.	1/6–22	Cortisone 1300 mg. (17d)	—	—	—	—	—	—	—	—	—	—				Constant edema since outset with no spontaneous diuresis.
Ht. 91.5 cm.	1/16		20.0	45.1	—	—	220	39.0	0.21	—	—	1.2				No diuresis following Cortisone.
Wt. 14.1 kg.	2/20		16.8	37.5	—	—	123	34.7	0.30	—	—	1.1				
S.A. 0.58M²	3/1–8	Cortisone 575 mg. (8d) DCA 40 mg.		—	—	—	—	—	—	—	—	—				No diuresis.
	3/29		22.5	—	—	—	—	—	—	—	—	—				
	3/29–4/8	ACTH 880 mg. (11d)		—	—	—	—	—	—	—	—	—				
	4/10		22.5	44.1	—	—	200	27.9	0.22	—	—	1.6				
	4/11		22.5	—	—	—	—	—	—	—	—	—				Diuresis 13th day after start of therapy (4/11).
	4/14		20.5	46.0	—	—	168	25.7	0.27	—	—	1.8				Minimum weight following diuresis.
	4/15		15.0	—	—	—	—	—	—	—	—	—				
	5/8		17.0	43.5	—	—	154	29.4	0.28	—	—	1.5				A second course of ACTH therapy (5/22–6/6 920 mg.) induced no diuresis.
	5/26		22.3	—	—	—	—	—	—	—	—	—				Generalized edema continues 8/17/50.
	8/7		25.5	—	—	—	—	—	—	—	—	—				
Expected normal value				*41.2*			*211*	*25.9*	*0.20*			*1.6*				
M. L.	3/2/50		20.7	29.5	—	—	170	20.7	0.17	—	—	1.4				Duration of disease—1 mo.
M. Age 3 yrs.	3/4		21.4	—	—	—	—	—	—	—	—	—				Constant edema with no spontaneous diuresis.
Ht. 91.5 cm.	3/4–13	ACTH 800 mg. (10d)	—	—	—	—	—	—	—	—	—	—				
Wt. 14.1 kg.	3/14		23.9	44.2	—	—	198	28.2	0.22	—	—	1.6				
S.A. 0.58M²	3/15		21.4	—	—	—	—	—	—	—	—	—				Diuresis 11th day after start of therapy (3/15).
	3/20		15.9	43.1	—	—	157	24.7	0.27	—	—	1.7				
	3/22		14.5	—	—	—	—	—	—	—	—	—				Minimum weight following diuresis.
Expected normal value				*41.2*			*211*	*25.9*	*0.20*			*1.6*				No manifest edema—8/17/50.
S. S.	2/27/50		15.0	29.0	—	—	241	16.0	0.12	—	—	1.8				Duration of disease—15 mos.
M. Age 3.5 yrs.	3/2–8	Cortisone 400 mg. (7d)	—	—	—	—	—	—	—	—	—	—				Constant edema since outset with no spontaneous diuresis.
Ht. 94.0 cm.	3/9		19.3	41.3	—	—	193	14.0	0.21	—	—	3.0				
Wt. 14.5 kg.	3/23		15.0	17.1	—	—	129	12.0	0.13	—	—	1.4				No diuresis after Cortisone.
S.A. 0.60M²	3/27		15.0	—	—	—	—	—	—	—	—	—				
	3/27–4/8	ACTH 1040 mg. (13d)	—	—	—	—	—	—	—	—	—	—				
	4/4		15.6	—	—	—	—	—	—	—	—	—				Diuresis 8th day after start of therapy (4/4).
	4/6		15.6	60.8	—	—	238	17.7	0.26	—	—	3.4				
	4/9		12.7	—	—	—	—	—	—	—	—	—				Minimum weight.
	4/11		13.2	34.3	—	—	211	26.1	0.16	—	—	1.3				
Expected normal value				*42.7*			*218*	*26.8*	*0.20*			*1.6*				No manifest edema 8/17/50.

in the C_{IN} : C_{PAH} ratio are associated with vascular changes or alterations in the permeability of the glomerular membrane cannot be assessed at present.

Maximum tubular excretion of PAH

Increases in T_{MPAH} accompanied increases in C_{IN} in two children (M. L. and S. S.) in whom it was measured before and during diuresis. The changes in T_{MPAH} were less than those in C_{IN} so that the C_{IN} : T_{MPAH} ratios increased from initially low values before to higher values. In the remaining child in whom repeated measurements of T_{MPAH} were made, no change in C_{IN} was observed and the changes in T_{MPAH} were irregular.

Plasma and blood volume

Increases in plasma volume during or after diuresis were observed in four out of five children in whom such observations were made. The changes in I. C., K. N., B. B., and J. S. were 29, 17, 26, and 11% respectively, above the control values. Corresponding increases in blood volume in I. C., K. N., and J. S. were observed but the changes were proportionately less because of decreases in hematocrit values. Failure to observe an increase in plasma volume in R. K. may possibly be explained again by a failure to make the measurement at the proper time

DISCUSSION

Prompt increases above initially reduced rates of glomerular filtration are commonly seen during the early stages of acute nephritis in children. On the other hand, reduced clearances in children with the nephrotic syndrome of several months' duration have been interpreted as indicating a less reversible process. The rapid increases in glomerular filtration rate reported here in children who had reduced kidney function and who were given ACTH demonstrate, therefore, a potentiality for improvement in function beyond that commonly considered possible in children with the nephrotic syndrome.

There is no evidence and it is not implied that these results are peculiar to diureses induced by ACTH. Although the magnitude of the changes was somewhat greater than those previously reported with other agents (1, 4, 19), it is entirely possible and probable that quantitatively similar changes may accompany spontaneous or induced diureses.

The results described have no bearing on the question of whether pituitary or adrenal cortical hormones have any direct relationship to the nephrotic syndrome. They do indicate that ACTH administration may induce repeated diureses at predictable times permitting serial measurements of changes which may accompany and be involved in the mechanism of diuresis. Thus large increases in glomerular filtration rate and significant increases in plasma volume were observed during diureses in these children. These observations lend support to the concept (20) that increases in plasma volume and in glomerular filtration rate accompany and may be involved in the mechanism of diuresis in children with the nephrotic syndrome.

SUMMARY

Profuse, sustained diureses occurring at predictable times in a high percentage of trials during or following administration of ACTH to children with the nephrotic syndrome permitted observations on changes which may accompany and be involved in the mechanism of diuresis. Serial measurements of discrete kidney functions and plasma volume are reported before, during and after 13 such diureses in eight children. The results indicate:

1. In five children with initially reduced rates of glomerular filtration, increases in inulin clearances ranging from 50 to 256% above control values were observed during or following diureses on five out of six occasions. In three observations on three children with rates of glomerular filtration initially within the normal range, an increase of 54% was observed in one. Thus in nine observations where inulin clearances were measured before and after the outset of diuresis, significant increases were observed in six.

2. Daily 24-hour creatinine clearances, measured in one of the three instances in which no marked increase in inulin clearance was observed *after* diuresis, suggest that glomerular filtration rate did increase *during* diuresis. In addition, the 24-hour clearances suggest that a marked increase in glomerular filtration rate during diuresis may be followed by a decrease from the maximum

value, but with subsequent stabilization at a value slightly or markedly above the control. Repeated responses in children with reduced function may then be associated with a progressive increase to normal or even "supernormal" values.

3. A consistent decrease in the endogenous creatinine: inulin clearance ratio was observed as glomerular filtration rate increased. However, clearances of thiosulfate and inulin showed reasonably good agreement at both high and low rates of glomerular filtration.

4. Clearances and maximum tubular excretion of p-aminohippurate increased during diuresis but proportionately less than inulin clearances. Consequently, increases in the C_{IN} : C_{PAH} and the C_{IN} : $T_{M_{PAH}}$ ratios were observed as rates of glomerular filtration increased.

5. In four of five observations on five children, increases in plasma volume (T-1824) ranging from 11 to 29% above control values were observed during diureses.

CONCLUSIONS

1. Diureses occurring during or following administration of ACTH to children with the nephrotic syndrome are usually associated with marked improvement in kidney function. Repeated diureses in children with initially reduced function may be associated with progressive increases in rates of glomerular filtration to normal values. These observations demonstrate a potentiality for improvement in function beyond that commonly considered possible in the nephrotic syndrome of many months' duration. It is believed that these changes are not peculiar to diureses associated with ACTH administration but may accompany spontaneous or other types of induced diureses.

2. The changes observed in plasma volume and glomerular filtration rate support the concept that increases in plasma volume and glomerular filtration rate are associated with the mechanism of diuresis in children with the nephrotic syndrome.

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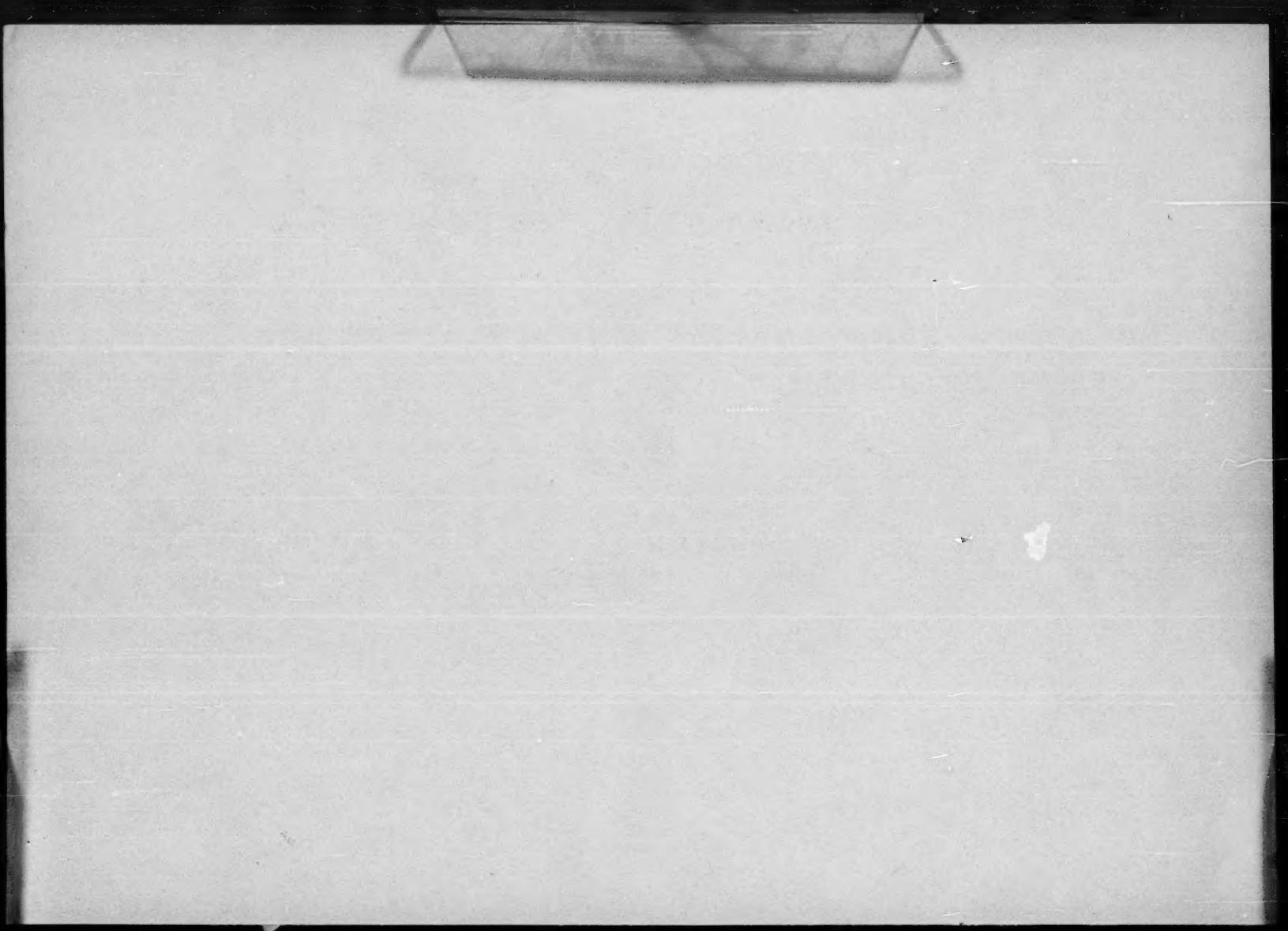

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